MANAGEMENT, EDUCATION AND BUSINESS STUDIES

IN a circular issued to local education authorities on March 28, 1960*, the Minister of Education gave details of the radical changes which are to be made in schemes of management studies and commercial education in Britain. Due to take effect in the autumn of 1961, the changes will affect both the existing courses leading to the Intermediate Certificate and Diploma in Management, which are administered jointly by the Ministry of Education, the British Institute of Management and other professional management bodies, and the Ordinary National and Higher National Certificates in Commerce, which are administered by a joint committee of professional bodies, local education authorities and the Ministry of Education.

The changes in management education and commercial studies have been announced jointly because, to some extent, future arrangements will lead to amalgamation of existing courses and to much more simplified administrative arrangements for the control of the new courses which have been devised.

The development of education for management in England and Wales is described in the Report of a Working Party which is issued as an appendix to Circular 1/60. Following the report of the Committee on Education for Management appointed by the Minister of Education in 1945 under the chairmanship of Colonel L. F. Urwick, a scheme of management studies was begun in senior technical colleges about nine years ago. The courses recommended were of five years duration and led to examinations for the Common Intermediate Certificate in Management Studies at the end of three years and a Diploma in Management Studies which was taken at the end of five years. Over the nine years the Urwick courses have led to considerably increased interest in management education, and industry in Great Britain owes a considerable debt of gratitude to Urwick and his committee for devising practical schemes of management studies which at long last the country is beginning to take seriously.

Inevitably the pioneering courses have met with criticisms and these have been considered by the Working Party which began its work in October 1955. The need to reconcile conflicting views of numerous professional management bodies, industrial and commercial organizations, educational institutions and local education authorities has caused the Working Party to tread a tortuous path and to take an unconscionable time in producing its report. This was submitted to the Minister in October 1958, and the changes now announced represent another triumph in producing an agreed scheme which should do much to put management education at a level where it can no longer be ignored by the purists or by those practical men who came up the so-called hard way.

At present there is a lack of adequate provision for extended management courses of an advanced nature suitable for men and women who are already professionally qualified, and there are relatively few colleges with a nucleus of full-time staff able to develop such courses and to engage in research. So far, too, management education has been conditioned by its history in that it has usually been closely associated with economics and commerce, and has left in relative neglect the social sciences, particularly psychology and sociology. The Minister is convinced that there is both an educational and also a pressing economic need for a major advance, and proposes to set up a course leading to a Diploma in Management Studies. This course may not, in general, be taken by people unless they already possess graduate or equivalent professional qualifications and have had a measure of relevant practical experience in employment. If taken part-time, the courses will last three years, with a minimum entry age of twenty-three. No diplomas will be awarded before the age of twenty-six.

Entry to courses will normally be permitted only to students who hold a degree, a diploma in technology, a final professional qualification approved for the purpose, or a higher national diploma, or people in managerial positions whose education has been interrupted since leaving school and who do not have the necessary academic entry qualifications. For those with appropriate experience exceptional admission will be available.

Experience in management studies has shown the advantage of full-time, and especially full-time residential, courses over part-time courses. Those advantages are especially apparent for students of mature age and experience and of proved academic ability. The Minister indicates that full-time residential courses will increasingly be found to constitute the most satisfactory way of meeting the needs of the students for whom the Diploma scheme is designed.

In the existing courses many of the students are young, inexperienced youths who seek to obtain managerial positions by long and arduous studies spread over at least five years of often badly taught evening classes; others are frustrated clerks and some of middle age who have been passed over for promotion, and who turn to management evening classes to prove themselves and restore their fortunes. Much misery has resulted from this system, and the Minister now roundly calls upon employers to remedy this unsatisfactory position by supporting part-time or full-time day or, even more desirable, full-time residential courses.

In principle, postgraduate courses of the type envisaged should be conducted only in colleges which are well equipped and staffed for the purpose and which offer courses in other subjects at postgraduate

* Ministry of Education. Circular 1/60 (28th March, 1960): The Future Development of Management Education and of Business Studies—Revision of Joint Examination Schemes. Pp. 3. Appendix to Circular 1/60: Report of the Working Party appointed to examine the Scheme of Management Studies. Pp. 1+57. (London: Ministry of Education, 1960.)

level as well as advanced courses in the separate aspects and techniques of management. So far as full-time and sandwich courses are concerned, the Minister recommends that provision should be restricted to the Colleges of Advanced Technology and those Regional Colleges which are already conducting well-established courses at the level of the Diploma in Management Studies. Only by such concentration will it be possible to build up the nucleus of full-time staff well qualified in the economic, physical and social sciences and able to conduct the research and consultancy work which are the essential bases of a lively postgraduate course. This long-overdue step will do much to improve the quality of management education.

Students of management would have been much happier with the Circular, however, if greater emphasis had been placed on the maintenance of adequate teaching standards at senior technical colleges by means of regular inspection. The Minister's statement would also have been improved if it had contained some reference to the well-known passage in the Urwick Report that "Theoretical Training alone does not make a Manager".

Besides the Diploma in Management Studies, Circular 1/60 also describes the setting up of two new National Certificates in Business Studies—an Ordinary and a Higher. These will replace the existing Common Intermediate Certificate in Management Studies as well as the Ordinary and Higher National Certificates in Commerce. Unlike the proposal to develop a new Diploma in Management Studies, which is likely to meet with the warmest support from thinking management in industry, the proposal to introduce Ordinary and Higher National Certificates in Business Studies may not meet with much enthusiasm. Ordinary and Higher National Certificates have acquired significance as qualifications pursued by enterprising youths and young men who attend part-time day and evening classes. The Ordinary and Higher National Certificates in Commerce have never acquired respectability, and to dissolve these into the new Ordinary and Higher National Certificates in Business Studies will give the latter emotional overtones which may take them a long time to throw off before becoming accepted as a genuine first step in management studies. An Ordinary National Certificate for genuine students of commerce should have been preserved. The Higher National Certificate in Commerce could well have been dropped, and a new certificate, similar to that proposed both by the Minister and the Working Party, should have been introduced. It should have no direct connexions with the Ordinary National Certificate in Commerce, the name "National" should not figure in it, and it should, as the Minister proposes, be available to all those students who at present take the Common Intermediate Certificate in Management Studies as well as students who hold Ordinary National Certificates in metallurgy, engineering, physics, commerce or any other discipline. The new Higher National Certificate in Business Studies will inevitably be regarded as the natural development from the Ordinary National Certificate in Business Studies. Its name may lead to its demise.

The third proposal contained in the Minister's circular is that an Advisory Council on Education for Management should be set up. Unlike the new diploma and certificates, which only apply to England and Wales, the proposed Council will be established by the Minister of Education, Secretary of State for Scotland and the Minister of Education for Northern Ireland, and will be concerned with management education in the United Kingdom. Such a Council is badly needed; it was one of the major recommendations in the Urwick Report. The three Ministers concerned are to be commended for its establishment, for its proposed composition, and its wide terms of reference. The Council will consist not only of representatives of appropriate educational institutions and of the major professional bodies concerned with management education but also representatives of those scientific and technological institutions which are themselves much interested in management education, together with representatives of both sides of industry and a number of other members with knowledge of management education generally, including management education in the universities. It will be concerned not only with the development of management education at levels beyond the equivalent of a university degree but also with the encouragement of research in management studies.

The new proposals should lead to a silent revolution in British industry. Despite all our success stories, those familiar with industrial undertakings are only too aware that the general picture is still one of muddle, mis-management and the rule of the thumb. Real work-study is little applied; budgeting is unknown; marketing is regarded as hocus-pocus; stock control using operational-research techniques is regarded as unsuitable for 'practical' men. A significant difference between American and British industry is that the Americans have long believed in the value of management education; the consequences are well known. Top management in British industry should now grasp the opportunities provided by the Minister to spread the scientific mode of thought along the lines advocated by Urwick and other pioneers many years ago. Now that the Minister has come down so heavily on the side of residential courses for management studies, perhaps he may soon announce what the Government proposes to do to fulfil its election promise of setting up an Advanced Centre for Management Education with university status. He should also ask his newly appointed Advisory Council to examine the existing facilities for management education to see if some order may not be built up from the existing confusion. The diversified activities at the Administrative Staff College, management consultant training centres, university and technical college management courses should be brought together to fit into a unified pattern of education for management. Existing residential courses of the requisite standard should be accepted in part for the new Diploma in Management Studies.

RUSSIAN APPROACH TO MINERAL PARAGENESIS

Physiochemical Basis of the Analysis of the Paragenesis of Minerals

By D. S. Korzhinskii. Translated from the Russian. Pp. 142. (New York: Consultants Bureau, Inc.; London: Chapman and Hall, Ltd., 1959.) 7.50 dollars; 63*s*. net.

THIS book, which was originally published in Russian in 1957, is an excellent, clearly written, introductory discussion of the application of thermodynamics and the principles of projective geometry to the analysis of the paragenesis of minerals in multi-component heterogeneous natural silicate systems.

The first chapter discusses the thermodynamic principles governing mineral paragenesis and is especially notable in two respects. First, by its consideration of the extensive parameters, that is to say, those such as mass, volume, entropy and enthalpy, the magnitudes of which depend on the size of a system or phase as distinct from the intensive parameters such as temperature, pressure and concentration. These extensive parameters have long been neglected even in classical thermodynamics and this neglect has been carried over into most of the thermodynamic discussions of natural silicate systems. Secondly, and equally notable, is the extension of the phase rule to cover open systems and not just isolated and closed systems, which are now generally recognized to be extremely rare in geological conditions. Apart from his memorable studies of the basement rocks of eastern Siberia, the author is perhaps best known for his views on metasomatism and the significance of 'perfectly mobile' components the chemical potentials, activities and concentrations (not masses) in one of the phases or partial vapour pressures of which are factors of equilibrium of a system. Without such a concept of 'perfectly mobile' components the thermodynamic approach to open systems is impossible. The author is careful to restrict his consideration of components to independent components, but we must hope he will eventually extend his treatment.

The second chapter deals with methods of representing the chemical composition of various systems, especially multi-component ones, with emphasis on the use of projective geometry in graphical methods. Of particular interest is the use of vectors to represent many (for example, seven) component compounds, following Lodochnikov's earlier work. All the constructions are clearly explained and fully illustrated with admirably labelled diagrams.

Chapter 3 concerns the relation between the chemical and mineralogical composition of rocks under constant external conditions, and includes examples of the paragenetic analysis of minerals in multi-component systems, while Chapter 4 considers the dependence of mineralogical composition on external conditions. In this section more examples are given, which include the use of determinants in calculations of reactions in multi-component systems. A short explanation of the routine in using determinants is given.

In the brief final chapter a few general comments emphasize that in most naturally occurring mineral assemblages the problem is to find the conditions under which the minerals crystallized, using the observed or deduced paragenesis, and that the determination of the stable assemblage in each of numerous different, but associated, rocks is important, but frequently difficult to achieve. No mention is made of the vital importance of synthetic laboratory studies.

Examples of the application of the author's approach to particular systems such as the five-component system, CaO-MgO-SiO_2-CO_2-H_2O, and the paragenetic analysis of granitic rocks in relation to the chemical potentials of sodium and potassium, both of which are fully examined, are particularly interesting, and more examples would have been generally welcomed. It is unfortunate that some of the examples do not use co-existing minerals actually analysed and that in the last-mentioned example average chemical compositions of biotites and hornblendes in rather different rocks, and rather poor averages at that, should have been used as the basis for the calculations. Integration of some of the experimentally determined laboratory results available with the natural parageneses could have been profitably included.

Perhaps most important of all, this book directs our attention to the woefully inadequate training in physical chemistry and mathematics of, unfortunately, nearly all geologists. The prevailing emphasis in most universities on laboratory and field studies, to the almost complete exclusion of theoretical geology, especially thermodynamics, has resulted in most geological research becoming a distressingly empirical subject of unco-ordinated fact-finding, and we must welcome this new and fundamental, even if introductory, synthesis.

BERNARD E. LEAKE

STEROLS AND TERPENES

Cholesterol

By Prof. David Kritchevsky. Pp. xi+291. (New York: John Wiley and Sons, Inc.; London: Chapman and Hall, Ltd., 1958.) 78*s*. net.

Ciba Foundation Symposium on the Biosynthesis of Terpenes and Sterols

Edited by G. E. W. Wolstenholme and Maeve O'Connor. Pp. xii+311. (London: J. and A. Churchill, Ltd., 1959.) 45*s*. net.

ACCORDING to a note on the dust cover, Dr. Kritchevsky's book "provides a centralized source of information on the biological function and significance of cholesterol. It covers cholesterol chemistry, biochemistry, and physiology". One must undoubtedly admire the courage of an author who is willing to undertake such a herculean task, especially when a rival work, which appeared almost simultaneously, required 15 co-authors. As a guide to the literature, it is of considerable value, for reference is made to approximately 2,000 publications.

The first chapter, which deals with the chemistry of cholesterol and its derivatives, is only partially successful. In the space of about 50 pages, the reader is led from the first studies of gallstones in the first half of the eighteenth century to total syntheses of steroids at the beginning of the second half of the twentieth century. The result of such compression is likely to be difficult for biologists to understand and of little value to the chemist. For example, the Woodward synthesis is covered in eight lines of text and a flow-sheet. Sarrett's original synthesis of 11-ketoprogesterone is mentioned, but subsequent

improvements (*J. Amer. Chem. Soc.*, **77**, 1026, 3824 (1955)) have not been included. Johnson's synthesis of *epi*androsterone is omitted entirely (*J. Amer. Chem. Soc.*, **75**, 2275 (1953)). This chapter closes with a useful account of methods of synthesis of isotopically labelled derivatives of cholesterol.

Knowledge of the biosynthesis of cholesterol has increased so rapidly that already the second chapter, through no fault of the author, is badly out of date. Realization that cholesterol biosynthesis proceeds through phosphomevalonic acid has come since the publication of this book. Details of the rearrangements which occur during the formation of lanosterol from squalene have also been elucidated recently.

During the remainder of the book, the author appears to be more at home with his subject, and there are successive detailed accounts of the absorption, transport, and metabolism of cholesterol, the implication of cholesterol in disease states, cholesterol-levels in the blood, and analytical methods for cholesterol. The overall impression which the reader is likely to gain from this part of the book is one of confusion. The author gives a detailed account of a mass of experimental results which abounds with apparent contradictions. Perhaps a more critical approach would have been better. Generous use of subheadings and tables, however, simplifies the reader's task to a considerable extent.

The book ends with a long and useful appendix, which contains a wealth of physical data on cholesterol, its derivatives, and related sterols. In addition, there are tables giving the cholesterol content of foods, sera, breast milk and tissues of many species. Typographical errors in structural formulæ were found on pp. 32 and 64.

The Ciba Foundation has a happy knack of organizing symposia in which the most eminent workers on biological subjects of great current interest are invited to contribute. The published records of these symposia are valuable both to those directly interested in the subject and to those who gamely continue the struggle to keep abreast of modern advances. The uninhibited discussions, which follow the individual contributions, enable the critical reader to appreciate divergencies of opinion among the experts, regions where hypotheses are ahead of experiment, and hence, the possible directions of future research. The present volume is no exception. The 17 papers, which comprise the symposium, cover most facets of the field of terpenoid and steroid biosynthesis. From acetate to cholesterol and the bile acids through such intermediates as mevalonic acid and its phosphate esters, squalene, and lanosterol, is a long story, but such has been the interest, particularly since the discovery of mevalonic acid, that most of the essential steps are now fairly clearly defined. The importance of acetate and mevalonate in the biosynthesis of triterpenes and carotenoids is clear, but these aspects of the field seem less fashionable than cholesterol, perhaps because of the clinical interest in the link between the biochemistry of cholesterol and atherosclerosis.

It is difficult to select any of the work reported in this volume for particular mention. One must, however, pay tribute to the painstaking work of Folkers' group; their discovery of mevalonic acid has provided a tremendous stimulus to biosynthetic studies in this field. For sheer brilliance of experimental technique, the elucidation, by Cornforth and his colleagues, of the mechanism of the rearrangement of methyl groups, which occurs during the cyclization of squalene to lanosterol, is difficult to surpass. The paper describing the biosynthesis of terpenoid compounds in fungi by Birch and Smith has an originality of ideas which stems from a combination of a wide knowledge of natural product chemistry and the mechanisms of organic chemical reactions. Thus, structures as different as mycophenolic acid, mycelianamide, gibberellic acid, and rosenonolactone are shown to be closely related biogenetically.

During the chairman's opening remarks and several times during the subsequent discussion, Sir Robert Robinson parades his anti-carbonium ion banner without seeming to intimidate all the authors, for a number of structures with integral positive charges on carbon are to be found within these pages. It should be remembered that this symposium deals necessarily with only a part of the story of the biosynthesis of terpenes and sterols, namely the nature of the reactions undergone by the substrates in the enzyme-controlled sequence which starts from acetate. Perhaps one day it will be possible to add the missing chapters and write out detailed reaction mechanisms, which include the parts played by the various enzymes, and then it may well be recognized that Sir Robert's criticisms are justified.

D. T. Elmore

STATISTICAL METHODS IN BIOLOGICAL AND MEDICAL SCIENCES

Statistical Methods in Biology

By Dr. Norman T. J. Bailey. (Biological Science Texts.) Pp. ix+200. (London: English Universities Press, 1959.) 25*s*. net.

Elementary Statistics with Applications in Medicine and the Biological Sciences

By Prof. Frederick E. Croxton. Pp. vii+380. (New York: Dover Publications, 1959.) 16*s*. net.

THESE books are intended for different audiences. Dr. Bailey's book "is to provide workers in the biological and medical sciences with an elementary account of the chief statistical methods liable to be needed in practice". It does this admirably, in a very readable manner.

One of the features of the book is a separate summary of statistical formulæ, arranged so that the reader may easily find the technique appropriate to his problem. In the main text Dr. Bailey is careful to underline the pitfalls surrounding each method, but for obvious reasons of space these do not reappear in the summary. At the beginning of the summary Dr. Bailey warns: "If there is any doubt as to the application or interpretation of any formula, the reader should refer back to the full discussion". The reader should act on this frequently.

The main part of the book covers the usual field of such a treatise—basic ideas of estimation and significance tests, standard distributions and tests, and first principles of experimental designs. Mathematical symbolism is kept to a minimum. As a result, many formulæ appear without introduction: but this is justified by the book's intentions. Curiosity about mathematical derivations may be satisfied elsewhere.

The biologist will find this book extremely useful, both as a 'field guide' and for an insight into the

methods employed in statistics. The statistician also would be advised to study this book, as an example of how lucidly statistical principles and arguments may be presented to non-mathematicians.

Dr. Bailey's book is not intended for undergraduate students as yet unfamiliar with research problems. It is to them that Prof. Croxton's book is directed. But as a whole, although the many excellent worked examples are medical in origin, the book is strongly biased towards methods more important to social scientists than to medical or biology students. The first third of the book is devoted to methods of presentation of data, and calculations of medians, modes, skewness and kurtosis. Thereafter the student is led through linear and non-linear correlation, (the word 'regression' appears only in the index), the normal, binomial and Poisson distributions, the reliability and significance of means and proportions, χ^2 tests, and finally a brief section on analysis of variance and allied procedures. As in Dr. Bailey's book, mathematical symbolism is avoided as much as possible; but replacing e by its numerical value in the normal distribution is surely carrying this 'simplification' too far. Prof. Croxton uses language and notation which is not standard, and some arguments, presented in general terms, are applicable only to the particular example under consideration. Although the book might be useful to a student of social medicine, it is on the whole unsatisfactory as an introduction to statistical methods as applied to experimentation.

Both books are attractively presented, Dr. Bailey's solidly bound, Prof. Croxton's in the well-constructed series of Dover reprints; and both are inexpensive by modern standards. R. M. CORMACK

ADVANCES IN PLANT PHYSIOLOGY

Annual Review of Plant Physiology

Vol. 10. Edited by Leonard Machlis, in association with John G. Torrey. Pp. vii+483. (Palo Alto, Calif.: Annual Reviews, Inc., 1959.) 7 dollars.

THE tenth annual review of plant physiology follows the same pattern as previous volumes in that no attempt is made to cover advances made in the whole range of plant physiology, but rather to concentrate on a number of restricted fields so as to give a picture of the present position of knowledge in them resulting from recent developments.

The subjects dealt with in the 19 articles which make up the present volume are diverse. Metabolism is well represented. Respiratory mechanism in higher plants, a subject which has attracted a great deal of attention in recent years, is dealt with by D. P. Hackett, and the closely related topic of the metabolism of carbon compounds by M. Gibbs. P. K. Stumpf and C. Bradbeer report on fat metabolism in higher plants, R. H. Burris on nitrogen nutrition and N. W. Pirie on leaf proteins. Two articles are concerned with chlorophyll-containing bodies, one by J. T. Wolken on the structure of the chloroplast, the other by A. W. Frenkel on reactions of bacterial chromatophores induced by light. Various aspects of mineral nutrition considered by W. H. Chandler, T. C. Broyer and P. R. Stout, and S. H. Wittwer and F. C. Teubner have a special bearing on horticulture and agriculture, while the article by G. G. Laties on active transport of salt into plant tissue deals with a fundamental problem of salt relations of plants.

As regards growth and development, J. Doorenbos and S. J. Wellensiek discuss the influence of photoperiod on floral induction, while two articles are concerned with growth-regulating substances, one on the chemical regulation of growth by a number of substances by F. C. Steward and E. M. Shantz, the other, by H. E. Gruen, on auxin and fungi. Phototropism and phototaxis are discussed by J. Reinert.

The remaining four articles are a miscellaneous assemblage, the subjects dealt with being lignins by R. E. Kremers, the pine tree by N. T. Mirov and R. G. Stanley, virus diseases by F. C. Bawden and plant chemotherapy by A. E. Dimond and J. G. Horsfall.

These articles are for the most part well written and generally avoid the fault of developing into a series of abstracts. They are highly informative and their production must have involved a vast amount of work on the part of the various authors. The high standard and usefulness of previous volumes is fully maintained in this latest annual review.

W. STILES

ROTATING FLUID MASSES

Figures of Equilibrium of Celestial Bodies, with Emphasis on Problems of Motion of Artificial Satellites

By Zdeněk Kopal. Pp. vi+135. (Madison: University of Wisconsin Press, 1960.) 3 dollars.

THIS important monograph is an exposition of the hydrostatic theory of self-gravitating fluid masses subjected to the distorting effects of rotation and tidal attraction. After a short introductory chapter, Prof. Kopal describes in Chapter 2 the first-order theory, due essentially to Clairaut, Legendre and Laplace, and discusses the mathematical properties of Clairaut's equation. The first-order theory assumes that the fluid mass departs from spherical symmetry by only a small amount, the square of which can be neglected. In Chapter 3 the theory is extended to cover the second-order effects of either the rotation of the body or the tides raised on it by a near neighbour; and in Chapter 4 the effects of interaction between rotation and tides are worked out. Second-order theory has previously been formulated by Callandreau, Darwin, de Sitter, Jeffreys and others; but Prof. Kopal's treatment is more general, and the contents of his Chapters 3 and 4 represent an impressive extension to the existing theory. The short Chapter 5, which concludes the book, discusses the free and forced oscillations of fluid bodies, with special reference to the possibilities of resonance.

The main application of the new theory is in double-star astronomy: in close binary systems the tidal effects in particular can be large enough to require a treatment correct to the second-order.

The subtitle of the book, "with Emphasis on Problems of Motion of Artificial Satellites", is most misleading, and would seem to have been added as an afterthought. These problems are not discussed, except in brief assertions on pages 4, 53 and 113 that the new theory will have an impact on Earth-satellite orbital studies. Since neither the solid Earth nor the atmosphere is exactly in hydrostatic equilibrium, and the Earth's surface is not an exact equipotential, it is

difficult to see how the new theory can be directly relevant to the study of Earth-satellite orbits, and more explanation would certainly have been welcome.

It cannot be pretended that the book is easy to read, though this is due partly to the difficulty of the theory. When the author has the chance to relax from the rigours of mathematics, as in the introductory paragraphs of each chapter, his writing is lucid and lively: the last paragraph on page 87 is particularly admirable in giving new life to a well-worn metaphor. The book is printed by a photographic method from a typescript. The resulting product is extremely clear, but the appearance of some of the formulæ would have been improved by more frequent use of small type, especially in fractions. Trivial errors and misprints are commendably rare, though equation (1–39) of Chapter 2 seems to be wholly erroneous and should be replaced by equation (2–3) of Chapter 3.

D. G. King-Hele

FROM NUCLEUS TO UNIVERSE

From Nucleus to Universe

A Course of Selected Lectures in Physics and Astronomy, University of Sydney, January 11–22, 1960. Edited by Prof. S. T. Butler and Prof. H. Messel. Pp. 335. (Sydney: Shakespeare Head Press, Pty., Ltd., 1960.) 84*s*.

THE excellent lectures delivered at the first of the summer schools for science teachers at the University of Sydney, sponsored by the Nuclear Research Foundation, were reviewed in these columns some two years ago (*Nature*, 182, 623; 1958) and have recently been re-issued by Messrs. Macmillan. This present volume contains those given at the third summer school of January 1960. They are of the same high standard, and given by a most distinguished group of scientists. The chief difference from the earlier volume is the thread of continuity that makes it more like a symposium on a single central theme.

The first contribution, by Prof. S. T. Butler, on introductory atomic and nuclear physics, deals with nuclear structure in general, and the interaction between charged particles and radiation. Next, Prof. C. N. Watson-Munro, on advances in thermonuclear research, discusses the physical conditions for maintaining a thermonuclear reaction and the orders of magnitude to be expected. The problems of stability in both linear and toroidal pinch machines, the field convolutions of the Stellerator type, the magnetic-bottle devices and the means of ion injection, are described with helpful diagrams; and there is a short summary of the means available for studying what goes on inside a plasma.

Prof. G. Gamow's account of the universe and its origin is the longest individual contribution. He discusses both the evolutionary and steady-state cosmologies, together with the kind of evidence which would help to discriminate between them. On the evolutionary theory, for the first tenth of the lifetime of the universe, radiation must have preponderated over matter; and the first critical stage from which the protogalaxies emerged came when matter began to play a significant part in affairs. This lively field of controversy was, it appears, submitted to American astronomers by the Gallup pollsters; unanimity was expressed in the answer to one question only—that asking whether such a poll was of any use! Prof. Bart J. Bok continues Prof. Gamow's story with an account of the observational basis for stellar evolution.

R. Q. Twiss, on stellar interferometers, describes the Michelson interferometer as originally used for the measurement of stellar diameters, and then shows how, as an 'intensity' interferometer, it was adapted to measurements on radio stars. The next stage in the development, which involved so much discussion a few years ago on the correlation of photons in two coherent interfering beams, was to bring it back as an intensity interferometer into optics. This work has led to a new type of interferometer, of which an account is given.

T. Gold discusses the origin of the solar system, the structure and evolution of the Earth, and the probable state of the surface of the Moon. A. A. Day, on the interior of the Earth, takes two models of the mantle-core arrangement and describes the physical conditions associated with each, mentioning the information expected to be given by the project of boring a hole down to the Mohorovičić-discontinuity. "Space Physics" is the title of D. F. Martyn's contribution, which describes the problems of launching satellites, the processes of observing and tracking and obtaining information from them, and the results of satellite observations. H. D. Rathgeber describes the Van Allen belt of radiation, and its probable part in the origin of the auroræ. G. B. A. McCusker gives an account of cosmic radiation, mentioning the association with solar flares, and describing some of the very high-energy nuclear events observed in photographic emulsions exposed to cosmic rays. The study of conditions in the upper atmosphere by observations of meteor trails, micrometeorites and the zodiacal light are among the topics mentioned by G. Elford in his account of the science of meteors. Finally, K. B. Mather writes of research in Antarctica during the International Geophysical Year, and of work on the structure of the Antarctic continent.

The original aim of the summer school was to give teachers the means of arousing enthusiasm for science among their pupils, and to stimulate the flow of entrants to the university science courses; and the 1958 publication was effectively a permanent record for the participants. This is a more luxurious production, which is intended for a wider circle of teachers, and could be understood by their more enterprising pupils or by the interested layman. I do not feel justified in complaining about the price, which seems fair for a handsome limited edition of real intrinsic value; indeed, it would be conniving at the major education heresy of our times to imply that one can expect to get what is first-rate on the cheap. At the same time, it is a pity that this book is out of the reach of the thousands of teachers who would read it with delight, and of most of the librarians who are working to a budget. There must be some way of meeting the latent demand and the not-so-latent need for such a book; and perhaps on a future occasion it may be found. For this is more than a shot in the arm for the jaded teacher whose equipment is a little obsolete; it embodies the new outlook, or the resurgence, of a science that is still an adventure of the human spirit rather than the mere handmaid of technology. If we are to begin to think seriously, as we shall surely have to, about science as a liberal study in schools, this is the kind of book that will direct our thoughts along the right lines.

G. R. Noakes

SPACE SCIENCE

THE First International Space Science Symposium held during the second week of January at Nice was attended by nearly two hundred delegates from all over the world. The meeting was organized by the International Council of Scientific Unions Committee on Space Research and was held in the Centre Universitaire Méditerranéen. In the foyer and on the landing was arranged an exhibition of instruments for space research with a display of some experimental results. A particularly strong contribution from the United Kingdom brought the work of this country for the first time before the eyes of the world.

During the first decade after the War geophysical and astronomical research by means of rockets, or space research as it has now come to be called, made important but for the most part undramatic progress. With the approach of the International Geophysical Year the rate of firings increased greatly as rockets were launched by way of a dress rehearsal for that period of intensive effort. During the period itself this research with rockets ceased to be the prerogative of the United States and the U.S.S.R., as they were joined by Australia, Britain, Canada, and Japan and later by France. In the meantime artificial satellites were launched, and scientists of several nations who did not have facilities for their own launchings joined in the tracking of these bodies and made use of their orbital behaviour to determine more precisely the figure of the geoid and to study the density of the atmosphere above 200 km.

The meeting at Nice was the first opportunity for a comprehensive presentation of the work being done in various parts of the world, and it provided, both in its formal sessions and in informal periods, time for the discussion which was now due. Much of the work had been carried out during the International Geophysical Year, but important results obtained since, especially with lunar and deep space probes, were also presented. Indeed, the success of the International Geophysical Year firings, together, it must be admitted, with an element of international competition between the U.S.S.R. and the United States, has ensured that more use than ever is being made of rockets for research.

Table 1 compiled from the Committee of Space Research reports on national activities gives an indication of the various national efforts. Only successful firings are included, that is those in which the original objective was largely achieved.

The rockets used were generally either of the 1 ton–150 lb. payload class (for example, the British *Skylark*) or of the multi-stage small solid-propellant type (for example, the American *Nike-Cajun*). Altitudes were generally around 150 km. However, the Russians used their geophysical rockets. These can carry payloads of about 2 tons and reach altitudes of more than 450 km. Frequently much of this payload was used for experimental animals. In terms of payload carried, therefore, their effort compares favourably with that of the United States. Aspect control in the Russian work has been achieved by spherical containers, weighing about 700 lb. ejected from the geophysical rocket. *Lunik III* was of similar form. The Americans have made good use of aspect control for solar spectroscopy by employing *Aerobees* fitted with the University of Colorado *Sun-Seeker*.

Table 1. VERTICAL SOUNDING

	Rockets fired above 100 km. in 1959	Artificial satellites up to Dec. 31, 1959	Space probes up to Dec. 31, 1959
U.S.A.	More than 36	4	1
U.S.S.R.	6	3	3
U.K.	7	—	—
Japan	4	—	—
France	3	—	—
Canada	1	—	—

Russian work has been characterized by a relatively high degree of standardization in the instrumentation (so that one type of instrument is flown again and again) and in the use of one rocket to carry many different experiments. On the other hand, the field of study of the Americans has been wider both as regards the phenomena investigated and the techniques used.

The hundred papers read at the conference were classified under seven headings. I shall endeavour to direct attention to some of the more important conclusions and results rather than to remark on individual papers.

In the opening session, on the Earth's atmosphere, Wexler discussed some of the doubts which have been current concerning the value of meteorological pictures made from satellites. He showed a film made during an *Atlas* firing which revealed a broad band of cloud associated with a front over the North Atlantic, the complex zone of the tropics and the organization of tropical clouds into streets, many of them aligned with the wind in the sub-cloud layer.

Important results obtained by the velocity of sound method in the Arctic, reported by Stroud *et al.*, showed the seasonal variation of wind and temperature. In the summer the prevailing winds were relatively gentle, usually less than 50 m./sec. from the east, while the temperature minimum at 80 km. was very deep, about 170° K. In the winter it was 60° warmer at this height and the winds were twice as fast and from the west. Two winter firings showed a breakdown of the circulation with strong northerly and southerly winds associated with sharp temperature increases in the stratosphere.

A comprehensive paper by Poloskov presented the Russian results on the neutral gas and the ionization. On the whole the data agreed well with the better-known American data. Mass spectrometer results showed the degree of dissociation of nitrogen up to 203 km. to be less than 2 per cent. Diffusive separation set in above 100 km., and the major positive ions found were atomic and molecular oxygen and nitric oxide.

In view of the importance of the ionosphere, and the amount of work done on it, the number of important results reported was remarkably small. Con-

spicuously lacking were data on sporadic and polar ionization, and on irregularities and motions. There was a tendency for the papers to deal with methods rather than results in this section. It is clear that Langmuir probes are emerging from the cloud of suspicion which has surrounded them since Langmuir's immediate successors found that their use was not always simple. Several of the papers were concerned with results from a study of satellite radio signals. Especially interesting are Garriott's results on the Faraday effect, which tend to confirm Evan's Moon echo measurements, that there are four times as many electrons above the *F* maximum as below. Thus above the maximum the concentration falls slowly. It was also found that magnetic activity could be accompanied by severe distortions of the profile, although the total content did not change.

The great importance of the discovery of the Van Allen belts of charged particles, trapped in the geomagnetic field, was reflected in the session on cosmic radiation and interplanetary gas. Eighteen papers were read, with important contributions from Van Allen, Vernov, Dolginov and Kursanova and their collaborators. Van Allen gave the flux of electrons in the outer zone of energy greater than 40 keV. as 10^{11} (cm.^{2} sec.)$^{-1}$. At 200 keV. this had fallen by three orders of magnitude. The proton flux in the outer zone was less than 10^{3} (cm.^{2} sec.)$^{-1}$ for particles more energetic than 60 MeV. The outer zone undergoes large fluctuations associated with solar activity, aurorae, atmospheric heating and magnetic storms. The inner belt is very constant in intensity and form and is thought to be due to decay of cosmic-ray neutrons. The electron fluxes are 100 times smaller than in the outer zone while the proton flux is about 100 times greater.

The Russian data agree fairly well with Van Allen's and extend the energy-range of the measurements. However, Vernov reported some apparent magnetic control of the inner zone intensity. In the same paper he gave the γ-ray flux in interplanetary space as less than 0·1 photon cm.$^{-2}$ sec.$^{-1}$ for energies greater than 450 keV., and less than 3 photons cm.$^{-2}$ sec.$^{-1}$ for energies greater than 45 keV.

Magnetic field measurements made by flux gate magnetometers were reported by Dolginov. These showed the existence of the field associated with the outer terrestrial radiation belt and also that the field of the Moon was less than 100γ. This gives a value for the lunar magnetic moment not more than $\frac{1}{4}$ per cent of the Earth's, in accordance with the idea that the Moon has no liquid core. The same general conclusion is indicated by the lack of evidence for a lunar radiation belt in the data obtained by *Lunik II*.

Cosmic ray measurements by *Lunik II* gave the following ratios for the fluxes of heavy rays, 1,000 : 75 : 3 for Z values 2 : 5 : 15. An interesting possibility suggested by some of these results, but not yet confirmed, is the trapping of α-particles in a radiation-belt situated 20,000 km. from the Earth's centre.

In the sessions on tracking and telemetry the paper by O'Keefe was of geophysical importance. Early studies of artificial satellite orbits gave a value of 1/298·3 for the equatorial bulge, which is about $1\frac{1}{2}$ per cent less than the internationally accepted value. These studies considered only the second harmonic, but in 1958 it was pointed out in Great Britain that the use of orbits at various inclinations would make it possible to determine higher harmonics simply from the precession-rates. On the other hand, it was found that a careful elimination of the effects of drag from the high satellite *Vanguard I* resulted in a motion containing two secular terms and four terms of period equal to the period of revolution of the line of apsides. Two of the periodic terms are interdependent, but the remaining five coefficients lead to values for the 2nd, 3rd, 4th and 5th harmonics. These results show that the shape of the geoid is not that which it would have if it were in rotational equilibrium. Indeed, it is not even symmetrical, as there is a significant 3rd (but not 5th) harmonic. O'Keefe considers the possibility that the distortion is due to convection currents, and rejects it on energetic grounds in favour of Jeffrey's model. He concludes that the Earth has considerable mechanical strength. It does not seem possible that the Earth's interior is a viscous fluid with a viscosity around 10^{22} poises as has been supposed.

The study of solar radiation is one of the most important branches of space science. As a sample star, close at hand, the Sun is of immense astrophysical significance, while its short-wave radiation is responsible for the ionosphere, with its varied structure and behaviour. The extensive and systematic work of the team at the Naval Research Laboratory, Washington, was among the most beautiful (if the term may be allowed) reported at the symposium. This team has used ionization chambers, high-resolution grating spectrographs and double-grating imaging monochromators to obtain valuable data on the emission spectrum, and to photograph the solar-disk in Lyman-α radiation. Good use has been made of the University of Colorado *Sun-seeker* to obtain details of the Lyman-α profile and to reveal strong reversal in the centre of the line.

Taking the American work as a whole (no work on solar ultra-violet or X-rays was reported from the U.S.S.R.) the outstanding results to date are summarized in the following quotation from a paper by Rense: "(1) Extension of the solar Fraunhofer spectrum to about 1500 Å. and the identification of over 1,000 absorption lines. (2) The detection of the prominent hydrogen Lyman-α line and the measurement of its absolute intensity. (3) The determination of the half-width and profile of the hydrogen Lyman-α line. (4) The detection of several members of the Lyman series and the continuum. (5) The detection of the ionized helium resonance line at 303·8 Å. and the establishment of its relatively high intensity. (6) The detection and wave-length measurements of several hundred emission lines extending from about 1900 Å. to 84 Å., with positive identification in many cases. (7) The measurement of the approximate absolute intensities of several of the less-intense emission lines. (8) Successful photography of the Sun's disk in hydrogen Lyman-α radiation".

The work on X-rays has concentrated chiefly on the effects of the flares. For flares in the range class 2–3 the flux between 20 and 100 Å. has averaged about 2 ergs cm.$^{-2}$. Between 8 and 20 Å. it was found to be about 3×10^{-2} erg. cm.$^{-2}$ and between 2 and 8 Å., about one-third of this value. Considerable hardening of the radiation thus occurs during such flares, together with a doubling of the total flux. Results suggest the existence of local thermal sources with a temperature of the order of 10^{6} ° C.

The session on the Moon and the planets contained a number of papers, reflecting the stimulation of interest in these fields by the rapid development of space technology. Of special note is the serious consideration now being given to space biology. The only rocket results reported in this session, however, concerned the now well-known *Lunik III* photograph of the reverse side of the Moon. It is a pity that the unique techniques employed in this superb piece of work were not discussed in greater detail.

R. L. F. Boyd

PRIMARY PROCESSES IN THE ACTION OF IONIZING RADIATIONS ON WATER: FORMATION AND REACTIVITY OF SELF-TRAPPED ELECTRONS ('POLARONS')

By Prof. JOSEPH WEISS

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During recent years, the study of the chemical action of ionizing radiation has been largely based on the hypothesis that the chemically active species are atoms and free radicals produced by radiation. This was introduced into the radiation chemistry of water, when it was suggested[1] that the absorption of the radiation leads to H atoms and OH radicals ($H_2O \longrightarrow\!\!\!\!\!\sim\!\!\!\!\!\sim\!\!\!\!\!\sim

leading to the formation of hydrogen atoms. There is, however, also some direct formation of hydrogen atoms, in a relatively low yield, from the dissociation of excited water molecules according to:

$$H_2O^* \rightarrow H + OH$$

The above considerations do not affect the secondary formation of H_2^+ ions[2] from H atoms and hydrogen ions ($H + H^+ \rightleftarrows H_2^+$) in relatively acid solutions.

In the presence of a suitable solute which can act as a polaron acceptor, this will also be competing for the polaron. For example, in the case of monochloroacetic acid, this reaction can be represented by[2]:

$$(H_2O)^- + Cl.CH_2.COOH \rightarrow Cl^- + CH_2COOH + H_2O \quad (5)$$

Other reactions are, for example, the reaction with hydrogen peroxide, according to:

$$(H_2O)^- + H_2O_2 \rightarrow OH^- + OH + H_2O \quad (6)$$

or

$$(H_2O)^- + O_2 \rightarrow O_2^- + H_2O \quad (7)$$

In solutions containing more than one acceptor one will, of course, have competition, as, for example, between reactions (3) and (7), respectively.

Another point of interest concerns the interaction of two polarons. It is well known that in the radiolysis of water a certain amount of molecular hydrogen is produced directly, presumably in the tracks of the ionizing particles. It has been thought previously that this may be due to the interaction of hydrogen atoms. However, such an assumption is not in agreement with many of the experimental facts. More recently[3], it has been suggested that this is due to the interaction of two of the ionic precursor species, which may lead to the formation of molecular hydrogen on one hand, and molecular hydrogen peroxide on the other hand, according to:

$$2(H_2O)^- \rightarrow H_2 + 2OH^- \quad (8)$$

$$2(H_2O)^+ \rightarrow H_2O_2 + 2H^+ \quad (9)$$

In view of the relatively great stability of the polarons, it is now perfectly conceivable that such bimolecular interactions may take place, particularly in regions of relatively high-ionization density.

It is interesting to look again at the physical situation: it is well known that the so-called F' centres can exist in an ionic crystal; these are believed to be two electrons occupying a negative vacancy. Such a configuration is stable and its stability is essentially due, in spite of the repulsion of the electrons, to the interaction of the electrons with the positive charges surrounding them. In fact, the net charge of the F' centre is the same as that of a mononegative particle. The situation is, however, different if there are no free positive charges present. In the case of a single polaron, the theory predicts considerable stability in a medium of high dielectric constant. However, for the same reasons one can show that what could be a 'bi-polaron' must be a highly unstable entity[4]: that is, if two polarons meet, they either repel each other or, if there is a possibility of a chemical interaction, mutual annihilation will occur.

Irradiated Ice

Some other indications for the existence of polarons can be derived from electron spin resonance measurements on irradiated ice and frozen acids (for example, sulphuric or perchloric acid). When acids are present in the ice, atomic hydrogen has been definitely established from observations of the hyperfine structure, and this was also confirmed by work on the D_2O-system[10]. On the basis of the above considerations, the formation of hydrogen atoms would be due to a capture of the polaron by the hydrogen ions of the acid according to equation (4). Such a view is to some extent supported by the fact that, in pure ice, no evidence for hydrogen atoms could be obtained[11]. A doublet and some other unresolved lines were observed after irradiation of pure ice, the former corresponding to a $g = 2{\cdot}0085$. Such a spectrum would not be incompatible with the presence of polarons, but cannot, of course, be regarded as a proof for these species.

When pure ice is irradiated, for example, with X-rays, a steady-state fluorescence and a weak, but reproducible, thermoluminescence is observed[12]. The fluorescence induced by irradiation of ice is suppressed in the presence of 2×10^{-5} M hydrogen peroxide, but on further irradiation the original luminosity is restored; this was found to be due to the gradual decomposition of the hydrogen peroxide during the irradiation. Presence of hydrogen peroxide likewise suppresses the thermoluminescence.

These phenomena, again, can be interpreted on the basis of the conversion of radiation energy into ionization and excitation.

Excitation in the solid may result in the formation of an 'exciton', and it is quite possible that the emission of fluorescence is, in fact, due to the radiative deactivation of 'excitons' in the ice.

On the other hand, ionization would lead to the formation of polarons: the observed decay of thermoluminescence, which is a first-order process and corresponds to a trap depth of about 0·3 eV., would be compatible with such an assumption. The trap depth of 0·3 eV. is, in fact, rather close to the value for the binding energy of the polaron in the ground state (H_0), which can be derived from Pekar's theory, who gives the expression[6]:

$$H_0 = -1{\cdot}47 \frac{\mu}{m} c^2 \text{ (eV.)} \quad (10)$$

Thermoluminescence could thus be due to the radiative annihilation of a positive and negative polaron according to reaction (3), or the radiative interaction of the polaron with an impurity centre. For water ($c^2 \simeq 0{\cdot}3$) and $\mu \sim m$, this gives a value of $H_0 = -0{\cdot}44$ eV.

I wish to thank Prof. N. F. Mott for his very helpful comments.

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EQUIVALENCE OF LIGHT AND ADENOSINE TRIPHOSPHATE IN BACTERIAL PHOTOSYNTHESIS

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PHOTOSYNTHETIC phosphorylation[1,2] has provided direct experimental evidence for the view that the key event in photosynthesis, the conversion of light into chemical energy, is independent of the classical manifestations of this process in green plants—oxygen evolution and carbon dioxide reduction. In the light-dependent phosphorylation of the cyclic type, that is the common denominator of photosynthesis in plants and bacteria, carbon dioxide is not assimilated nor is oxygen evolved; the only identifiable chemical product of the absorbed light energy is adenosine triphosphate[3,4].

In photosynthesis of green plants the contribution of light is not limited to the synthesis of adenosine triphosphate, but also includes the formation of reduced triphosphopyridine nucleotide[3]. Adenosine triphosphate and reduced triphosphopyridine nucleotides, jointly called assimilatory power[5], are then used for carbon dioxide assimilation. All the reactions of carbon dioxide assimilation occur in the dark and are also present in non-chlorophyllous cells[6-9] and must therefore be considered as being removed from the energy conversion process peculiar to photosynthesis. The validity of this concept of photosynthesis has been documented in isolated chloroplasts by separating experimentally their light and dark reactions, and by characterizing their carbon dioxide assimilation[10-13].

We have recently reported that, in the presence of hydrogen gas, the contribution of light in bacterial photosynthesis may be limited to the formation of adenosine triphosphate[3]. Similar views have also been expressed on the basis of independent evidence by Stanier, Doudoroff, Kunisawa and Contopoulou[14]. The reductant required for carbon dioxide assimilation, reduced pyridine nucleotide, is, unlike in green plants, formed in the *dark* by the external hydrogen donor, molecular hydrogen[3,4]. It follows, therefore, that if light is replaced by exogenous adenosine triphosphate, photosynthetic carbon assimilation should proceed entirely in the dark.

The substitution of adenosine triphosphate for light would be particularly significant if it could be demonstrated in such photosynthetic bacteria as *Chromatium*, that are unique in the living world in being strict phototrophs. *Chromatium*, unlike, for example, *Chlorella*, or photosynthetic bacteria of the genus *Rhodospirillum*, cannot replace its light-dependent mode of life by a heterotrophic, aerobic metabolism in the dark[15-17]. *Chromatium* grows only in the light[15,16], and being an obligate anaerobe, cannot carry out oxidative phosphorylation.

The purpose of this article is to report evidence which we have now obtained for the equivalence of light and adenosine triphosphate in the photoassimilation by *Chromatium* of carbon, supplied either as carbon dioxide or acetate. The findings to be reported support the view that bacterial photosynthesis is the prototype of photosynthesis in green plants, where it became modified in the course of biochemical evolution, to include oxygen evolution and the formation by light of a reductant for carbon dioxide assimilation[3].

Carbon Assimilation by Whole Cells

Until recently, the prevailing view of carbon assimilation in photosynthetic bacteria was that these organisms resemble green plants in forming most of their carbon compounds by reducing carbon dioxide. It was known for a long time that *Chromatium* can assimilate organic substrates in light[16]; but, in the main, these came to be regarded as hydrogen donors, rather than as sources of organic carbon for further photosynthetic elaboration into cellular material[17]. Stanier *et al.*[14] have now reopened this subject by showing that in "resting" cells of *Rhodospirillum rubrum* "acetate is converted anaerobically in the light largely to poly-beta-hydroxybutyrate". We have therefore re-examined the assimilation of carbon under different conditions using 'growing' *Chromatium* cells.

As shown in Table 1, significant fixation of carbon dioxide, labelled with carbon-14, by *Chromatium* cells occurred, as expected, only in the light and was greatly increased by the addition of malate, succinate, thiosulphate, sulphide, and molecular hydrogen. Acetate was an exception; it did not increase fixation of carbon dioxide.

The assimilation of acetate acquired special interest when we found, in parallel experiments, that *Chromatium* was able to grow with acetate as the sole source of carbon without added carbon dioxide. *Chromatium* has been grown with acetate previously[16] but always in the presence of added carbon dioxide. Since acetate did not aid the assimilation of labelled carbon dioxide (Table 1) and supported growth without the addition of carbon dioxide, it might have served as

Table 1. Effect of Light and of Inorganic and Organic 'Reductants' on Carbon-14 Dioxide Assimilation by *Chromatium* Cells

Treatment	Total $^{14}CO_2$ fixed (counts × 1,000/min.)
1. No 'reductant' added*, dark	80
2. No 'reductant' added, light	270
3. Acetate, light	270
4. Malate, light	630
5. Succinate, light	1,000
6. Hydrogen, light	1,320
7. Thiosulphate, light	1,020
8. Sulphide, light	1,040

* In the dark, no increase in carbon dioxide fixation was observed on adding any one of the 'reductants' listed in treatments 3–8.

Each vessel contained a modified culture solution (ref. 26) and cells equivalent to 0·2 mgm. bacteriochlorophyll; also the following in μmoles: *Tris*, pH 7·8, 60; carbon-14 bicarbonate, 10; 'reductant', 10 (except in treatment 6). Final volume 2·5 ml. Gas phase was argon, except in treatment 6, which received hydrogen gas. The reaction was carried out at 25° C. and stopped after 30 min. by adding 0·1 ml. 5 *N* hydrochloric acid.

Table 2. Effect of Light and Cyanide Inhibition on Carbon Assimilation by *Chromatium* Cells

Treatment	Total carbon-14 fixed (counts × 1,000/min.)
1. Carbon-14 acetate, dark	8
2. Carbon-14 acetate, light	480
3. Carbon-14 acetate, light	520
4. Carbon-14 acetate, 10^{-3} *M* cyanide, light	520
5. Carbon-14 dioxide, hydrogen, light	3,200
6. Carbon-14 dioxide, hydrogen, 10^{-3} *M* cyanide, light	1,500

Prior to the experiments the cells in treatments 1–4 were grown with acetate as the 'reductant' and the cells in treatments 5 and 6 with hydrogen gas (ref. 26). Treatments 1 and 2 were run for 20 min. and included, in each reaction vessel, cells equivalent to 0·13 mgm. bacteriochlorophyll; final volume 1·9 ml. Treatments 3–6 were run for 30 min. and included cells, equivalent to 0·2 mgm. bacteriochlorophyll, and 3 μmoles carboxyl-labelled acetate; final volume 2·5 ml. Gas phase was argon, except in treatments 5 and 6. Other conditions as in Table 1.

a direct source of carbon, but one that in *Chromatium* cells could be assimilated only in light.

We tested this interpretation by supplying radioactive acetate in the light and in the dark to 'growing' *Chromatium* cells in short-term experiments. The results are shown in Table 2. No significant assimilation of labelled acetate occurred in the dark. In the light a marked incorporation of acetate took place and was not affected by the addition of carbon dioxide.

Table 2 also presents evidence that the photoassimilation of acetate occurred by a pathway independent of that of carbon dioxide assimilation. We have previously reported that, in chloroplasts, the reductive carbohydrate cycle for carbon dioxide assimilation is inhibited by cyanide[12]. Table 2 shows that, in *Chromatium* cells, cyanide inhibited the assimilation of carbon dioxide, but not that of acetate. It seems, therefore, that carboxydismutase, which is the enzyme of the reductive carbohydrate cycle that

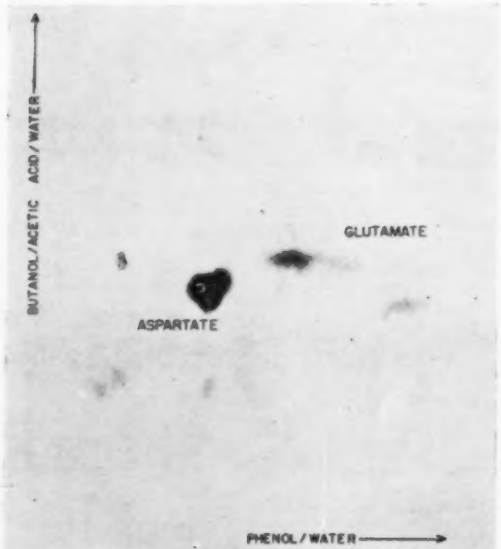


Fig. 1. Radioautograph of a chromatogram showing soluble products of carbon-14 dioxide assimilation in the light by *Chromatium* cells. Experimental conditions as in treatment 5 of Table 2, except that thiosulphate was used instead of hydrogen

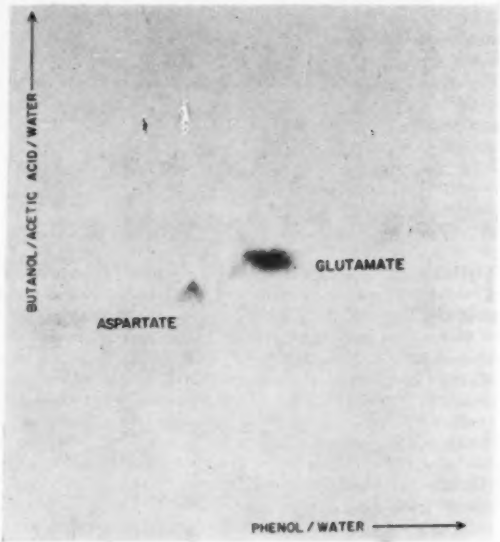


Fig. 2. Radioautograph of a chromatogram showing soluble products of carbon-14 acetate assimilation in the light by *Chromatium* cells. Experimental conditions as in treatment 3 of Table 2

is specifically inhibited by cyanide in *Chromatium* and in chloroplasts[12], is not involved in the photoassimilation of acetate by *Chromatium*. Acetate was apparently used by *Chromatium* cells not as a reductant in carbon dioxide assimilation but as a carbon compound that was readily photo-assimilated into cellular substance without external carbon dioxide and without being first oxidized to carbon dioxide.

The conclusion that carbon dioxide and acetate represent two independent pathways for carbon assimilation in *Chromatium* cells was strengthened by a comparison of the products formed. The identification was made by the techniques of paper chromatography and radioautography[11]. When labelled carbon dioxide was the sole source of carbon, and the reductant was either molecular hydrogen, thiosulphate, sulphide, malate or succinate, the main soluble products formed (approximately 50 per cent of the total carbon fixed) were in all cases amino-acids, principally aspartic acid.

A typical chromatogram is shown in Fig. 1. In general, phosphorylated sugars were much less prominent in *Chromatium* than in isolated chloroplasts[11–13] and in certain experiments no sugars were detectable by the identification techniques which we used. The prominence of aspartic acid among the products of carbon dioxide fixation is in agreement with the report of Fuller[13] that "in *Chromatium* the primary pool of fixed carbon is aspartic acid".

With radioactive acetate the products of photoassimilation in the soluble fraction (about 10 per cent of the total carbon fixed) were again mainly amino-acids, but here glutamate was the principal product formed (Fig. 2).

To recapitulate, then, carbon dioxide and acetate assimilation seemed to proceed in *Chromatium* by two separate pathways that had a common link in being strictly dependent on light. It now remains to be shown that, in both pathways, light is replaceable by exogenous adenosine triphosphate.

Carbon Dioxide Assimilation by Cell-free Preparations

The equivalence of light and adenosine triphosphate might have been directly demonstrated in whole cells if it were possible to accumulate or introduce substrate quantities of adenosine triphosphate for driving carbon dioxide fixation in the dark. However, a preliminary accumulation of adenosine triphosphate in the light is not possible because whole cells have only catalytic amounts of adenosine diphosphate. The introduction of exogenous adenosine triphosphate is also excluded by the impermeability of whole cells to adenylates. Experimentally, therefore, the problem is how to overcome the permeability barrier of the cells to adenylates without damaging excessively the photosynthetic apparatus.

In the case of green plants the permeability barrier of whole cells was set aside by using broken chloroplasts[19]. In the case of *Chromatium* the cells were ground with alumina and the cell-free fluids were separated by differential centrifugation into chromatophores that contained the photochemical apparatus proper, capable of photosynthetic phosphorylation outside the cell[19-21], and into a supernatant fluid which contains the carbon dioxide fixing enzymes[22]. The role of light and adenosine triphosphate in carbon assimilation by the cell-free preparations was investigated in parallel experiments using either carbon dioxide or acetate as the source of carbon.

Carbon dioxide assimilation in cell-free preparations of *Chromatium* seemed to proceed, as was the case in broken chloroplasts[12], by the reductive carbohydrate cycle. However, in the case of *Chromatium*, phosphoglycerate was the product that was being removed from the cycle and converted into phosphoenolpyruvate, which is the substrate of a second carboxylation reaction resulting in the formation of oxalacetate and aspartate. The enzyme that catalyses this carboxylation, phosphoenolpyruvate carboxylase, is also present in chloroplasts[23], but there, on present evidence, it does not seem to play as direct a part in photosynthetic carbon dioxide assimilation.

In the presence of exogenous ribulose diphosphate or phosphoenolpyruvate, cell-free preparations of *Chromatium*, like whole cells, assimilated carbon dioxide mainly into aspartic acid without any further requirement for energy either as light or as adenosine triphosphate. The dependence for either light or exogenous adenosine triphosphate became apparent, however, when ribulose diphosphate or phosphoenolpyruvate were replaced in the reaction mixture by ribose-5-phosphate or pyruvate.

Typical results, shown in Table 3, indicate that in the presence of ribose-5-phosphate, a marked increase

Table 3. Effect of Light and Adenosine Triphosphate on Carbon-14 Dioxide Fixation by Cell-free Preparations of *Chromatium* in the Presence of Ribose-5-phosphate

Treatment	Total $^{14}CO_2$ fixed in soluble compounds (counts × 1,000/min.)
1. Dark, control	54
2. Dark, ATP, R-5-P omitted	48
3. Dark, ATP	138
4. Light, control	54
5. Light, ADP + P	108

Each vessel contained in a final volume of 1·5 ml.: cell-free extract, containing 0·3 mgm. bacteriochlorophyll and the following in μmoles: *Tris*, pH 7·8, 80; cysteine 20; magnesium chloride, 5; manganese chloride, 2; potassium chloride, 20; carbon-14 bicarbonate, 10; ribose-5-phosphate (R-5-P), 5; and, when added, adenosine diphosphate (ADP), 0·5; orthophosphate (P), 5; and adenosine triphosphate (ATP), 4. Other experimental conditions as in Table 1.

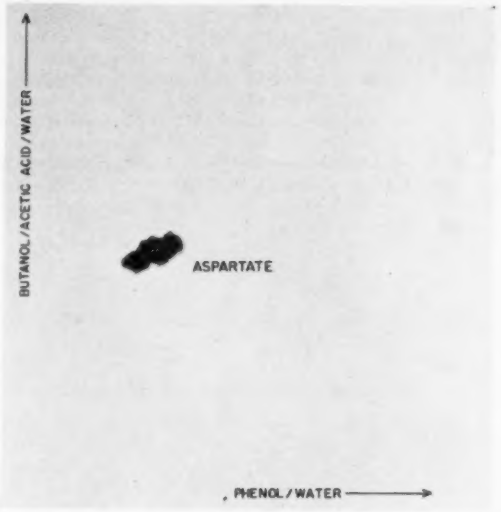


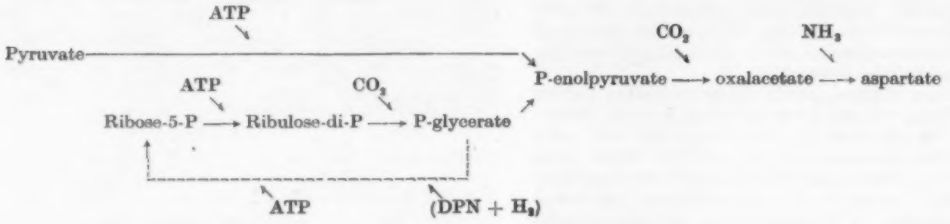
Fig. 3. Radioautograph of a chromatogram showing the soluble products of carbon-14 dioxide fixation in the *dark* by a cell-free preparation of *Chromatium* in the presence of ribose-5-phosphate and added adenosine triphosphate. Experimental conditions as in treatment 3 of Table 3. The same pattern of carbon dioxide fixation was obtained in the light without added adenosine triphosphate

in carbon dioxide fixation was observed in the light when adenosine diphosphate and inorganic phosphate were added to the reaction mixture. The addition of adenosine diphosphate made photosynthetic phosphorylation by chromatophores possible and thus supplied adenosine triphosphate to the reaction mixture. In the dark, a similar increase in carbon dioxide fixation occurred on adding exogenous adenosine triphosphate. The main product of carbon dioxide assimilation, aspartate, was the same in the light as in the dark with exogenous adenosine triphosphate (Fig. 3).

The equivalence of light and adenosine triphosphate was also observed when pyruvate was used as a carbon dioxide acceptor. The bacterial preparations were found to have a very active enzyme catalysing an exchange reaction (cf. ref. 24) between the carboxyl group of pyruvate and carbon-14 dioxide. The exchange reaction itself was independent of light or adenosine triphosphate, but carbon-14 dioxide fixation into aspartate and other dicarboxylic acids occurred only when light or adenosine triphosphate was present. For example, in the presence of pyruvate, carbon-14 dioxide, and exogenous adenosine triphosphate the main radioactive compounds formed (Fig. 4) were aspartate and pyruvate (by the exchange reaction). Other identifiable compounds were alanine, malate and fumarate. The formation of malate was in agreement with the presence in *Chromatium* extracts of the malic enzyme reported by Sisler and Fuller[25] and confirmed by us in other experiments[26].

A 'photosynthetic' pattern characteristic of *Chromatium* was also obtained without either light or exogenous adenosine triphosphate, in the presence of 3-phosphoglycerate or phosphoenolpyruvate as carbon-14 dioxide acceptor. The prominence of aspartate as the product of carbon-14 dioxide assimilation via the phosphoenolpyruvate carboxylation is illustrated by Table 4.

The pathway of carbon dioxide assimilation in *Chromatium*, which is now documented by these and other experiments[26] with cell-free preparations, either in light or in dark with exogenous adenosine triphosphate, is summarized below. The diagram shows the regeneration of ribose-5-phosphate from phosphoglycerate. We found in *Chromatium* extracts an active diphosphopyridine nucleotide-linked triose phosphate dehydrogenase, the enzyme which accounts for the reduction of phosphoglycerate that is involved in this step.



Assimilation of Acetate by Cell-free Preparations

The assimilation of acetate by cell-free preparations confirmed and extended the conclusions drawn from the acetate experiments with whole *Chromatium* cells. As shown in Table 5, in the presence of coenzyme *A*, acetate assimilation was greatly increased by light and by the addition of acetate 'acceptors', namely, oxalacetate or pyruvate.

Table 6 shows that the role of light was specifically concerned with the formation of adenosine triphosphate. Acetate assimilation occurred also in the dark, but only when exogenous adenosine triphosphate was added. No significant assimilation occurred when adenosine triphosphate was either omitted or when it was made unavailable for acetate assimilation by being trapped, in the light or in the dark, by a hexokinase–glucose system. These results, as well as the response from added coenzyme *A*, suggest that the role of light in acetate assimilation by *Chromatium* is to provide adenosine triphosphate that is required for the formation of acetyl–coenzyme *A*.

In cell-free preparations, as in whole cells, acetate was assimilated to glutamate. The investigation of glutamate synthesis from acetate revealed the presence in *Chromatium* of certain new synthetic pathways that have not been found in chloroplasts. *Chromatium* preparations, like chloroplasts (unpublished data), are able to activate acetate by converting it to acetyl-coenzyme *A*, and condensing acetyl-coenzyme *A* with oxalacetate to give citrate. Citrate is then converted via *iso*citrate and α-ketoglutarate to glutamate. Unlike chloroplasts, *Chromatium* preparations also condense acetyl–coenzyme *A* with glyoxylate[16,25] and, as we have now found with α-keto acids, pyruvate (reaction 1) and its analogue, α-ketobutyrate (reaction 2):

pyruvate + acetyl coenzyme *A* → citramalate (methyl malate) (1)

α-ketobutyrate + acetyl coenzyme *A* → ethylmalate* (2)

* The compound has been isolated but not yet unequivocally identified.

Table 4. Proportion of Aspartate in Soluble Products of Carbon-14 Dioxide Assimilation by Cell-free Preparations of *Chromatium*

Treatment	Per cent of $^{14}CO_2$ fixed as aspartate
1. Dark, pyruvate	15·7
2. Dark, pyruvate, ATP	50·1
3. Light, pyruvate, ADP + P	69·5
4. Dark, phosphoglycerate	40·0
5. Dark, phosphoenolpyruvate	81·0

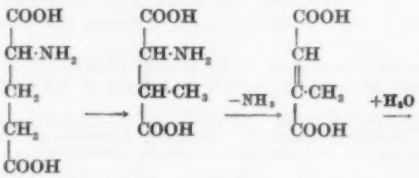
The reaction mixture common to all treatments was that given for treatment 4 in Table 3, except that ribose-5-phosphate was omitted and the following additions were made (in μmoles) where indicated: pyruvate, 5; phosphoglycerate, 5; phosphoenolpyruvate, 4; adenosine triphosphate (ATP), 4; adenosine diphosphate (ADP), 0·5; and orthophosphate (P), 5.

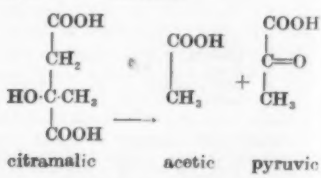
Table 5. Effect of Light, Oxalacetate and Pyruvate on the Assimilation of Carbon-14-acetate by Cell-free Preparations of *Chromatium*

Treatment	Carbon-14 fixed in soluble compounds (counts × 1,000/min.)
Dark, control	5
Light, control	42
Dark, oxalacetate	42
Light, oxalacetate	300
Light, control	27
Light, pyruvate	75

The reaction mixture common to all treatments was that given for treatment 1 in Table 3, except that ribose-5-phosphate and carbon-14 bicarbonate were omitted and 0·3 μmole of coenzyme *A* and 3 μmoles of carboxyl-labelled carbon-14 acetate was added. 10 μmoles each of oxalacetate or pyruvate was added where indicated.

Of the newly found condensations, the one of special interest was that of citramalate since Barker *et al.*[25] have shown that in *Clostridium tetanomorphum*, another obligate anaerobe, the citramalate pathway provides a mechanism for the degradation of glutamate to pyruvate and acetate:





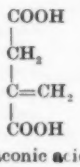
It now appears that in *Chromatium* the reversal of the degradative citramalate pathway may lead to

Table 6. EQUIVALENCE OF ADENOSINE TRIPHOSPHATE (ATP) AND LIGHT IN THE ASSIMILATION OF CARBON-14-ACETATE BY CELL-FREE PREPARATIONS OF *Chromatium*

Treatment	Carbon-14 fixed in soluble compounds (counts × 1,000/min.)
1. Dark, control	27
2. Dark, ATP	180
3. Dark, ATP, hexokinase	186
4. Dark, ATP, hexokinase, glucose	6
5. Light, control	414
6. Light, hexokinase	348
7. Light, hexokinase, glucose	20

Experimental conditions as in the oxalacetate treatments in Table 5. 1·5 mgm. hexokinase, Type III (Sigma Chemical Co.) and 10 μmoles glucose were added where indicated.

glutamate synthesis. Among the products of pyruvate condensation with acetyl-coenzyme *A*, we have identified citramalate and glutamate and another compound, which at first seemed to be mesaconate, but turned out to be the closely related itaconic acid (it may be noted that the R_F value for citramalate resembles that of compound *X* which Glover *et al.*[29] reported as the main product of acetate assimilation in *R. rubrum*):



itaconic acid

When oxalacetate was the acetate acceptor, the main products, that is, citrate and glutamate, were the same in light as in the dark with added adenosine triphosphate (Fig. 5). With pyruvate as the acetate acceptor the main products of acetate assimilation, either in light or with added adenosine triphosphate in the dark, were citramalate and glutamate (Fig. 6).

The reactions of carbon assimilation in *Chromatium*, for which there is now experimental evidence, are summarized in Fig. 7. Most of the component reactions have already been discussed. In this scheme the only contribution of light (except perhaps in the photo-production of hydrogen[30]) for both carbon dioxide and acetate assimilation is in the formation of adenosine triphosphate. Carbon dioxide assimilation is shown in Fig. 7 to be dependent on exogenous hydrogen, whereas the assimilation of acetate by the citrate or citramalate pathway indicated yields hydrogen for metabolic purposes in accordance with the overall equation 3 or 4:

$$3\ \text{acetate} + NH_3 \xrightarrow{5\ ATP} \underset{\text{(citrate pathway)}}{\text{glutamate}} + CO_2 + 6H \qquad (3)$$

$$3\ \text{acetate} + NH_3 \xrightarrow{4\ ATP} \underset{\text{(citramalate pathway)}}{\text{glutamate}} + CO_2 + 6H \qquad (4)$$

Further evidence for the reactions shown in Fig. 7 will be given elsewhere[26].

Concluding Remarks

The similarity between the carbon assimilation patterns of cell-free systems supplemented with adenosine triphosphate in the dark, and those found in whole cells of *Chromatium* in the light, provides, in our judgment, significant evidence that in this obligate phototroph exogenous adenosine triphosphate is equivalent to light. The role of adenosine triphosphate, whether formed by photosynthetic phosphorylation or supplied from without, was twofold. Adenosine triphosphate was either required for the formation of an 'activated' substrate (ribulose diphosphate, phosphoenolpyruvate or 1,3-diphosphoglycerate) for a subsequent carboxylation or reduction, or it was required for the formation of an 'activated' carbon source, as by forming acetyl-coenzyme *A* from acetate and coenzyme *A*. The activated compounds are then ready for participation in the synthetic reactions that are catalysed by specific enzyme systems, all of which function in the dark.

In this view of photosynthesis, carbon dioxide assimilation, although quantitatively the dominant form of photosynthesis on the Earth, is fundamentally only a special case of the use of light energy. It was previously suggested[5] that even in green plants, light energy, when converted to adenosine triphosphate, may be used for driving endergonic reactions other than carbon dioxide reduction. Experimental evidence

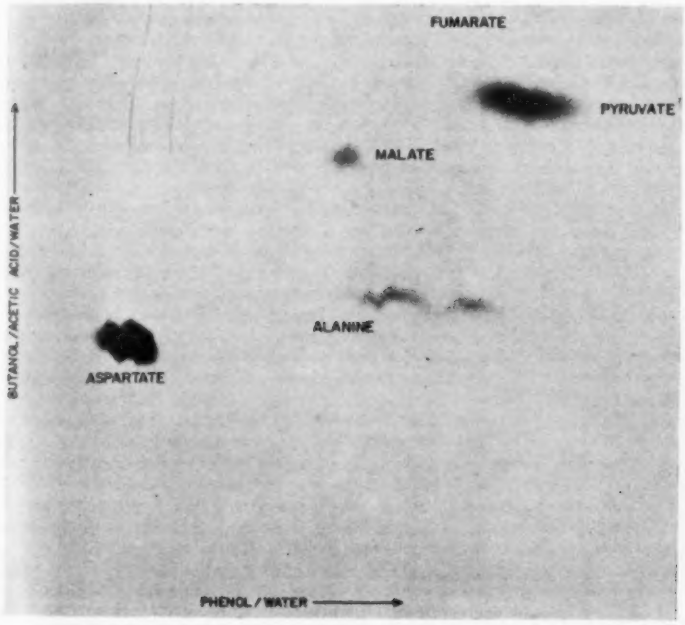


Fig. 4. Radioautograph of a chromatogram showing soluble products of carbon-14 dioxide fixation in the *dark* by a cell-free preparation of *Chromatium* in the presence of pyruvate and added adenosine triphosphate. Experimental conditions as in treatment 2 of Table 4. The same pattern of carbon dioxide fixation was obtained in the light without added adenosine triphosphate

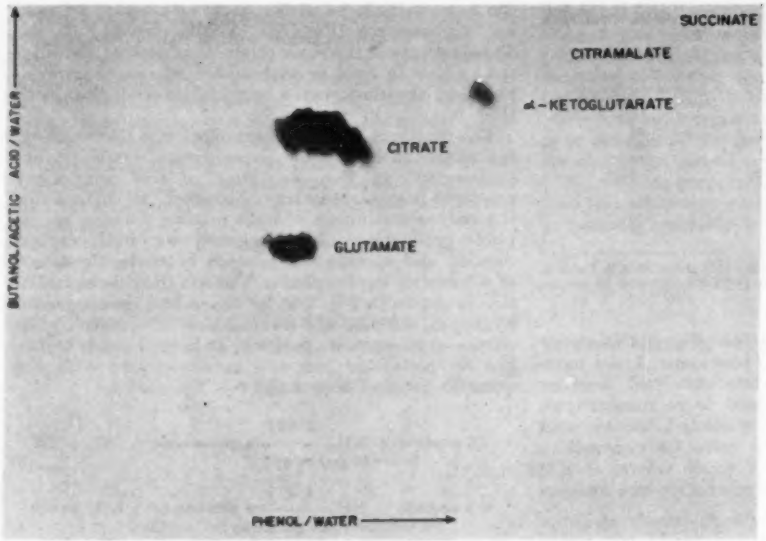


Fig. 5. Radioautograph of a chromatogram showing products of carbon-14-acetate assimilation in the *dark* by a cell-free preparation of *Chromatium* in the presence of adenosine triphosphate, coenzyme *A* and oxalacetate. Experimental conditions as in treatment 2 of Table 6. The same pattern of acetate assimilation was obtained in the light without added adenosine triphosphate

for this has recently come from the work of Maclachlan and Porter[21].

In the case of photosynthetic bacteria, carbon dioxide assimilation may, in certain circumstances, not even be the principal pathway for using the converted light energy for the synthesis of organic compounds (cf. ref. 14). As was shown here, *Chromatium* is able to assimilate acetate directly without an external source of carbon dioxide, either with the aid of light or with exogenous adenosine triphosphate. In this instance, therefore, the photo-assimilation of acetate is a case of photosynthesis without either carbon dioxide assimilation or oxygen evolution. In the conventional view of photosynthesis this state of affairs would be inadmissible, but according to the present concept the photo-assimilation of acetate by *Chromatium* constitutes photosynthesis because it involves biochemical syntheses that are being driven by light energy after it has been converted into adenosine triphosphate.

Carbon dioxide assimilation, in any event, in both green plants and photosynthetic bacteria, consists of exclusively dark reactions that are not peculiar to photosynthesis. It was already anticipated by Benson and Calvin, that "it is not to be expected that this type of work (investigation of the carbon cycle) will lead directly to a solution of the unique problems of photosynthesis, namely, the knowledge of the act or acts by which electromagnetic energy is transformed into chemical energy"[22]. The discovery of photosynthetic phosphorylation has since provided evidence that this transformation of energy is more closely linked with phosphorus than with carbon assimilation.

The photo-assimilation of acetate by *Chromatium* with the aid of adenosine triphosphate formed by light supports the suggestion made earlier[5], that, if in the early period of evolution of life forms, organic compounds were abundant and the Earth's atmosphere was a reducing one[23], then the light-dependent mechanism for adenosine triphosphate formation was

Fig. 6. Radioautograph of a chromatogram showing products of carbon-14 acetate assimilation in the *dark* by a cell-free preparation of *Chromatium* in the presence of adenosine triphosphate, coenzyme *A* and pyruvate. Experimental conditions as in treatment 2 of Table 6, except that pyruvate (5 μmoles) replaced oxalacetate, the gas phase was air, and chromatophores were removed from the cell-free preparation by centrifugation. The same pattern of carbon-14 acetate assimilation was obtained in the light, in the presence of chromatophores, without added adenosine triphosphate

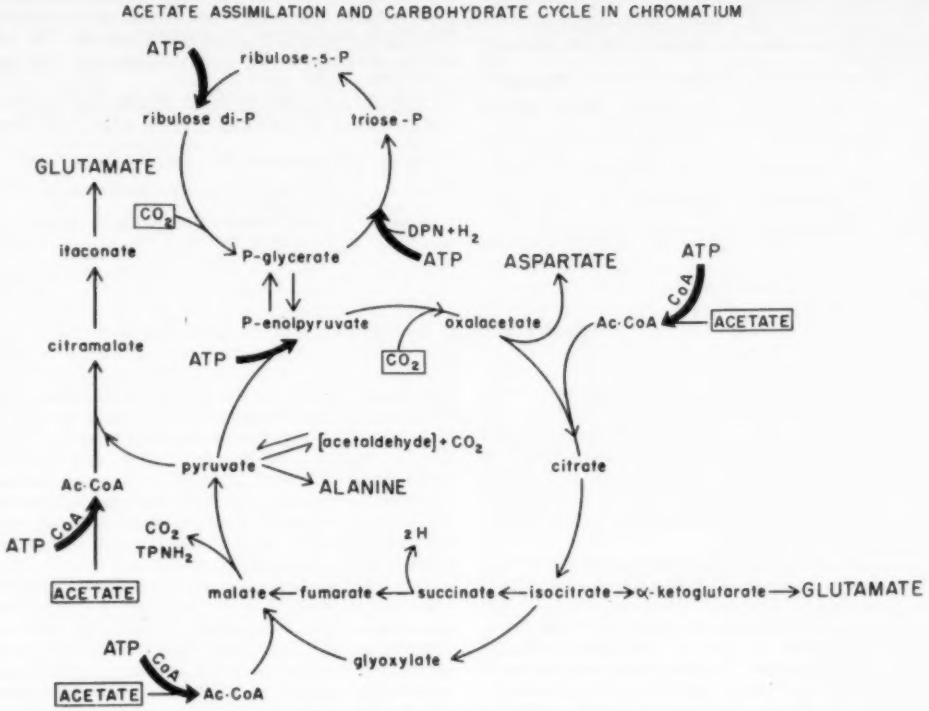


Fig. 7. Reactions of carbon assimilation in *Chromatium*. Further details are given in the text

the most important event for the newly emerging photosynthetic organisms. In this view, an obligate anaerobic phototroph such as *Chromatium* would represent the most primitive type of photosynthesis, one in which no oxygen is evolved, organic compounds serve as carbon sources and the main contribution of light is the formation of adenosine triphosphate. This primitive type of photosynthesis, it seems reasonable to suppose, was paralleled by transitional types in which the contribution of light was still the formation of adenosine triphosphate, but carbon dioxide was an important source of carbon, and the carbon dioxide reductant, reduced pyridine nucleotide, could be formed by dark reactions as, for example, with hydrogen gas. Finally, in the most advanced type of photosynthesis, carbon dioxide became the sole carbon source and the function of light was to provide both adenosine triphosphate and reduced pyridine nucleotide. Only in the last case, typified by green plants, when photosynthesis became strictly dependent on water for pyridine nucleotide reduction by light did oxygen evolution form an inseparable part of photosynthesis, as is envisaged by the electron-flow mechanism proposed elsewhere[3].

It would thus appear that oxygen evolution, which gave Priestley[24] almost two hundred years ago the first clue to the existence on this planet of what we now call photosynthesis, is, in the evolutionary time scale, the most recent aspect of the process (cf. van Niel, ref. 17). Photosynthetic bacteria, which have greatly contributed to our understanding of photosynthesis by showing that this process can occur without oxygen evolution[17], now also provide us with evidence that photosynthesis can occur without carbon dioxide assimilation. What remains fundamental to all photosyntheses is the conversion of light into chemical energy. This conversion appears to be intimately bound to photosynthetic phosphorylation and its product adenosine triphosphate, which is the universal 'energy currency' of all cells, whether they live in the light or in the dark.

This investigation was aided by grants from the National Institutes of Health and the Office of Naval Research.

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OBITUARIES

Prof. W. E. Garner, C.B.E., F.R.S.

The news of the death of Prof. W. E. Garner at his home in Bristol on March 4 was received with much sadness by his former colleagues at the University of Bristol, and particularly by those who had the privilege of working with him and under his leadership.

Garner was a man of great charm and kindness who won the affection and admiration of all who came into contact with him. His unselfishness and sincerity made him the most likeable of men. He was quiet, unobtrusive, entirely devoid of personal ambition and devoted to his work, whether it be in his laboratory, the councils of the University, or in the committee room. But these unassuming qualities did not obscure the greatness of the man. He was an enthusiastic and inspiring leader of research: keenly critical in scientific matters, yet very kindly and tolerant in his attitude to the ideas of others. Always most conscientious in the discharge of his duties, he maintained the tradition of lecturing to first-year students and he liked to know the students personally. The wide circle of friends, students and of many others who sought his advice and received it, valued a friendship which will endure as a cherished memory of a lovable character.

Garner was a student of the University of Birmingham, where he carried out research with Prof. P. F. Frankland. He was awarded an 1851 Exhibition in 1913 and proceeded to the University of Göttingen to work with Prof. Gustav Tammann. His work there was interrupted by the outbreak of the First World War, and on his return to England he worked at Woolwich Arsenal with Sir Robert Robertson on the calorimetry of high explosives.

On leaving Woolwich, Garner was appointed assistant lecturer in the University of Birmingham (January 1919) and assistant lecturer at University College, London (October 1919). From that time began a close and happy association with Prof. F. G. Donnan and a fruitful period of research.

In 1927, Garner was appointed to the Leverhulme chair of physical and inorganic chemistry in the University of Bristol, a post which he held until his retirement in 1955.

During the Second World War he established an extra-mural research team in the University of Bristol under the Ministry of Supply to assist Government ordnance factories in research on explosives and munitions. The group continued until the end of the War, although Garner himself moved to Fort Halstead in Kent to become in turn superintendent of Chemical and Explosives Research, deputy chief, and later chief, superintendent of Armament Research in the Ministry of Supply. His wide knowledge, enthusiasm and wise guidance were of paramount importance to the war effort, and he was associated with many important developments in armaments and munitions. His services were recognized in 1946 when he was made C.B.E.

On the cessation of hostilities, Garner once again returned to Bristol and continued his fundamental researches with increasing vigour. He had a wide interest in, and keep knowledge of, many branches of physical chemistry, and his mental qualities were only fully employed when he was simultaneously developing many research lines. Broadly, his main contributions to physical chemistry fall into the following groups: (*a*) The study of explosive reactions in gases and the ionization and radiation from flames; notable among these researches was the discovery of the effect of small amounts of hydrogen on the radiation from carbon monoxide flames. (*b*) Heterogeneous catalysis and particularly the measurements of heats of adsorption. He and his colleagues in Bristol made outstanding contributions to the mechanism of adsorption on metals and metallic oxides, and he was a leader in applying the newer ideas of the physics of the solid state to the interpretation of heterogeneous reactions. (*c*) The kinetics of solid reactions. Garner's pioneering studies on the mechanism of the interface reactions and nucleation processes in solid reactions, and the development of suitable experimental techniques, constitute a chapter to the progress of a subject in which he was the acknowledged leader in Britain.

After his retirement in 1955 his continued interest in these topics and his freedom from other responsibilities enabled him to organize a symposium on chemisorption at Keele, Staffordshire, which was published by the Chemical Society, and to edit a large volume on the "Chemistry of the Solid State". Most of the contributors to the latter volume were his former students or close scientific colleagues, and the book is very much a tribute to his work and inspiration.

Garner was a well-known figure at scientific gatherings, and scientific societies and Government committees made great calls upon his time. He was president of the Faraday Society (1945–47); a member of the Scientific Advisory Council of the Ministry of Supply for most of the period 1942–60, and also a scientific advisor for civil defence in the south-west region of Britain.

He received many honours : he was elected to the fellowship of the Royal Society in 1937 ; and was a Fellow of University College. He was well known abroad ; he was an honorary member of the Polish Chemical Society, a corresponding member of the Patronato "Alfonso el Sabio", Madrid (1959), and a member of the Joint Services Mission to the United States and Canada in 1948.

C. E. H. Bawn

Prof. A. J. H. Goodwin

Astley John Hilary Goodwin was born in Pietermaritzburg on December 27, 1900, and died on December 5, 1959, after a long illness. The son of Canon W. A. Goodwin, he was educated at St. John's College, Johannesburg, and at the University of Cambridge, where he graduated in archæology. At the age of twenty-three he joined the staff of the University of Cape Town, serving there until his death. He was appointed associate professor in 1954 in recognition of his academic achievements and original work.

From the sixties of the nineteenth century, collectors had amassed considerable quantities of stone implements from large numbers of sites in southern Africa. Goodwin, on his return from Cambridge, first applied his mind to the problem of the systematic classification and chronological arrangement of this material and soon realized the need for a terminology that would be more applicable to the southern African facts than the European one hitherto accepted. He envisaged a subdivision of the Stone Age into three—Earlier, Middle and Later—each of which embraced a number of cultures and their variations. Collaboration with van Riet Lowe and Neville Jones led to the foundations of South African prehistoric classification being laid and to the elaboration of conjoint ideas in "The Stone Age Cultures of South Africa". This contained basic systematic descriptions and has been used as the foundation for prehistoric studies in both South and East Africa.

Goodwin stressed the need for a strictly scientific approach to field exploration and to the drawing of conclusions. His method of approach is to be found in his handbook entitled "Method in Prehistory". His own field-work was intensive and marked by meticulous accuracy of observation, whether it was concerned with the excavation of caves and shelters or with the examination of sequences of styles in rock engravings. Mainly undertaken within the confines of the Cape Province, his original field observations were supplemented by visits to many critical sites in other parts of Africa ; and he excavated and studied important more recent archæological sites in Nigeria on the invitation of the Government of that country.

Goodwin's methodical and lively mind embraced many cultural interests and made him a good organizer. He took a leading part in founding the South African Archæological Society, was its secretary for a number of years, edited its *Bulletin*, and at the time of his death was its president. With A. L. du Toit he was enthusiastic in founding the Athenæum Trust, which provided a 'home' for the scientific and cultural societies, the headquarters of which are in Cape Town. For some years he served as the honorary general secretary of the Royal Society of South Africa and on its council and, again in the year of his death, was elected to its presidency. Whatever task of this nature he was called upon to perform, he carried it out with quiet unobtrusive natural efficiency and forthrightness ; what he had to say was said quietly, succinctly and directly. Discussion with him was a pleasure, but acrimonious discussion was impossible. To all those who had contact with him he was "John".

Our late colleague is survived by his widow, a son and a daughter. S. H. Haughton

Prof. C. J. Bakker

Prof. C. J. Bakker, whose death in an aeroplane accident in New York occurred on April 23, was on his way to attend a meeting of the American Physical Society in Washington. He had been invited to address the Society on recent researches at CERN and particularly on the successful operation of the 25-GeV. proton synchrotron.

Cornelis Jan Bakker was born in 1904 and studied physics under Zeeman in Amsterdam. After taking his doctorate (*cum laude*) in 1931 for original research on the Zeeman effect in the spectra of the noble gases, he spent a year in London at the Imperial College of Science and Technology continuing his work in spectroscopy. In the following year he joined the scientific staff of Philips, Eindhoven, where he worked on the physical problems of radio communications. His interests soon turned to nuclear physics and during the War, in collaboration with Prof. Heyn, he designed the Philips cyclotron. In 1946, he succeeded Gorter as professor of physics and director of the Zeeman Laboratory of the University of Amsterdam. He also became director of the Institute of Nuclear Physics, the focal point of Dutch nuclear research sponsored by Fundamental Onderzoek der Materie, by the City of Amsterdam and by Philips. He was a member of the Royal Netherlands Academy of Sciences.

In 1951, Prof. Bakker was invited by Prof. Auger, director of the Unesco Department of Natural Science, to be one of the eight experts charged with drawing up plans for the future European Organization for Nuclear Research (CERN).

At the first session of the Council of CERN during May 5–8, 1952, Prof. Bakker was appointed a member of the directorate and director of the Synchro-Cyclotron Division. On July 1, 1955, he became deputy director-general of CERN, succeeding Prof. Felix Bloch as director-general on September 1, 1955.

During the years 1955–60 CERN has grown from an idea put forward by a few European scientists to one of the best-equipped laboratories for high-energy nuclear physics research in the world. In 1957 the CERN 600-MeV. synchro-cyclotron was commissioned, and since that time it has been in full use as a nuclear physics research tool. More recently, at the end of 1959, the 25-GeV. proton-synchrotron came into operation at CERN.

In June 1957, Prof. Bakker received an honorary doctorate from the University of Geneva, and in April 1960 the Queen of the Netherlands awarded him the high honour of "Ridder in de orde van de Nederlandse Leeuw". His death took place before the official ceremony at which this award was to have been given.

Prof. Bakker believed firmly in European collaboration in science, and CERN, which is a unique and successful example of such collaboration, was inspired by his devoted leadership. His sudden death is a tragic loss to his wife and children, to CERN and to international scientific collaboration.

J. B. Adams

NEWS and VIEWS

J. H. Plummer Professorship of Experimental Physics at Cambridge : Prof. A. B. Pippard, F.R.S.

Dr. A. B. Pippard, who has been elected to the John Humphrey Plummer professorship of experimental physics in the University of Cambridge, went to the Royal Society Mond Laboratory immediately after the Second World War, and in a series of elegant experiments on the high-frequency behaviour of metals at very low temperatures laid the foundations of much of his subsequent work. Observations on metals in the superconducting state led him to postulate a 'non-local' relation between superconducting current and magnetic field, which proved very suggestive in the later development of a fundamental theory by Bardeen, Cooper and Schrieffer. The study of normal metals brought not only a detailed understanding of the 'anomalous skin effect', first noticed by H. London, but also led Pippard to consider other aspects of the theory of metals, and he has recently been particularly concerned with the relation between the electronic structure of a metal and its physical properties. All his work has been distinguished by a remarkable combination of experimental skill with an ability to see his way through complicated theory in terms of simple physical concepts. This has enabled him not only to devise ingenious experiments but also to contribute effectively in fields which are usually regarded as the preserve of the professional theoretician. He was elected a Fellow of the Royal Society in 1956 and was awarded the Hughes Medal of the Society in 1959; his election to the John Humphrey Plummer chair at Cambridge is a well-merited recognition of his outstanding position in metal physics.

Physics at Southampton : Prof. G. W. Hutchinson

Dr. Hutchinson, who has been appointed to a newly established second chair of physics in the University of Southampton, was educated at Abergele Grammar School and at St. John's College, Cambridge. After graduating in physics, he spent five years in industry and then, in 1947, started research in the Cavendish Laboratory under Prof. O. R. Frisch. He first investigated the properties of liquid argon as a conduction counter for nuclear particles, and, as a result, became interested in the analysis of pulse-height distributions. After trials of a number of systems Hutchinson realized the possibilities of computer techniques in this field, and working with G. G. Scarrott, devised in 1950 the pulse-height analyser which now bears their names. This elegant and powerful instrument represented a major advance in nuclear counting technique, and its widespread use was fitly recognized by the award of the Duddell Medal of the Physical Society to Hutchinson and Scarrott jointly in 1959.

Since 1952 Hutchinson has been Fellow and lecturer in the Department of Natural Philosophy, University of Glasgow, and lecturer and senior lecturer in the Department of Physics, University of Birmingham. His research interests have ranged widely, and include nuclear reactions at low energies, the proton–proton interaction at 1 GeV. and the origin of cosmic rays. Recently, he has been responsible for the experimental programme of the Birmingham proton synchrotron, and has developed techniques of fast counting and new forms of Čerenkov counter which have proved valuable for work with this machine. Hutchinson has always wished to extend his work through contact with the National Institute for Research in Nuclear Science, and his appointment to a chair in the University of Southampton at this time should help him to further his interests in high-energy physics.

The Advanced School of Automobile Engineering, Cranfield : Prof. J. R. Ellis

The governing body of the College of Aeronautics has appointed Mr. John Romaine Ellis as professor of automobile engineering and first director of the Advanced School of Automobile Engineering being established at Cranfield in Bedfordshire, in association with the College of Aeronautics. Mr. Ellis graduated in mechanical and aeronautical engineering in the University of London in 1947, and was awarded a master's degree in 1954. He became an associate member of the Institution of Mechanical Engineers in 1952 and of the Automobile Division in 1958. During the period January 1944–December 1948, Mr. Ellis was employed successively at the Royal Aircraft Establishment and the Fairey Aviation Co., Ltd. In 1949 he joined the lecturing staff of the Royal Military College of Science, Shrivenham, first in the Materials and Structures Branch, and is at present principal lecturer in the Machines Branch. His published papers have dealt with wind-up in multi-axle drive vehicles, the effect of speed of rotation and torque on the effective rolling radius of a pneumatic tyre, and with torque converters. He is at present investigating the steering mechanics of the pneumatic tyre.

Chemistry at Queen Mary College, London : Prof. M. J. S. Dewar, F.R.S.

After eight years as head of the Department of Chemistry at Queen Mary College, London, Prof. M. J. S. Dewar was appointed to the chair of organic chemistry in the University of Chicago, in October 1959. His stimulating personality and exceptionally wide scientific interests, ranging from physical theory to organic synthesis, produced striking developments in both teaching and research at Queen Mary College. Outstanding among his achievements, which led to his election to the fellowship of the Royal Society earlier this year, are his contributions to the molecular orbital theory of conjugated molecules, the synthesis of tropylium salts and new hetero-aromatic boron compounds, and the development and use of radio-spectroscopic techniques for nuclear quadrupole, electron spin, and nuclear magnetic resonance. His departure is a great loss to chemistry in Great Britain, but every good wish goes with him for success and happiness in the United States.

Prof. B. C. L. Weedon

Dr. B. C. L. Weedon has been appointed to the chair of organic chemistry at Queen Mary College, where he will take up his duties in October 1960. Dr. Weedon graduated with first-class honours at the Imperial College of Science and Technology, London, in 1942 and, after a year's research, joined the Dyestuffs Division of Imperial Chemical Indus-

tries, Ltd. In 1947, he returned, as a lecturer in organic chemistry, to the Imperial College where he collaborated with the late Sir Ian Heilbron and afterwards with Sir Patrick Linstead. He was awarded the Meldola Medal of the Society of Maccabæans and the Royal Institute of Chemistry for 1952, and became reader in organic chemistry in 1955. Dr. Weedon is well known for his distinguished work in the fields of natural and synthetic polyenes, carotenoids and vitamin A, terpenes, acetylenes, fatty acids, pigments and other constituents of algae, fungi and bacteria, and the reactions of organic compounds in molten alkali and at electrodes. He is co-author, with Sir Patrick Linstead, of "A Guide to Qualitative Organic Chemical Analysis", and this year became scientific editor of *Pure and Applied Chemistry*, a journal to be published by the International Union of Pure and Applied Chemistry. Dr. Weedon will be a source of great strength to the Chemistry Department at Queen Mary College in the period of expansion and development which undoubtedly lies ahead.

Commonwealth Scientific and Industrial Research Organization (Australia) Division of Entomology

Dr. Douglas F. Waterhouse has been appointed chief of the Commonwealth Scientific and Industrial Research Organization (Australia) Division of Entomology in succession to Dr. A. J. Nicholson, who has occupied the post for twenty-seven years. The Division of Entomology was one of the first Divisions formed when the Council for Scientific and Industrial Research was set up in 1926. Dr. Nicholson has been appointed to a C.S.I.R.O. research fellowship and will continue in the Division of Entomology his notable research in the field of animal populations. He was a foundation Fellow of the Australian Academy of Science and a member of its first Council, and has held the offices of secretary (1954–55) and vice-president (1955–57).

Dr. Waterhouse graduated with first-class honours and the University Medal from the University of Sydney in 1937. During the War he held the rank of captain in the Australian Army Medical Corps. He was awarded the degree of D.Sc. from his University in 1952, and in the following year he shared the David Syme Research Prize with Dr. F. J. P. Dwyer. He has made two trips overseas in connexion with his research work in insect physiology and toxicology, spending six months in Cambridge just after the War, and six months in New Haven, U.S.A., during 1955–56.

Movements of Scientific Men

In a written answer in the House of Commons on May 19, the Minister of Education, Sir David Eccles, as representing the Minister for Science, stated that the Department of Industrial and Scientific Research is at present undertaking, in co-operation with industry, a series of combined technical and economic studies of particular industries of national importance designed principally to discover where additional scientific research and development effort can most usefully be applied. A question was also asked referring to the emigration to the United States of another member of the scientific team working the Jodrell Bank radiotelescope. Sir David stated that as regards investigation into the reasons for emigration of scientists, he had nothing to add to replies given on March 28 and April 14, when he had pointed out that the general problem had been under consideration for some years by the Advisory Council on Scientific Policy, and had stated that, while there were no complete statistics of scientists who had emigrated to the United States or Canada, in 1958 7 scientists were recruited in America by the Civil Service Commissioners and the Atomic Energy Authority and in 1959, 16 were so recruited. Many scientists go overseas purely for further education, and others obtained valuable experience with which they later returned to Britain. In Sir David's opinion, a scientist should not be denied such opportunities, nor does it seem feasible for Britain to provide in every field of science, facilities identical with those in the United States.

Industrial Research in the United States

The final results of surveys, conducted for the National Science Foundation by the United States Department of Labour, Bureau of Labour Statistics, of funds and personnel for industrial research and development in 1956 present a picture generally very similar in character to that shown in Britain by the recent report from the Department of Scientific and Industrial Research (Science and Engineering in American Industry—Report on a 1956 Survey. Pp. ix+117. (NSF 59-50.) (Washington, D.C.: Government Printing Office, 1960.) 70 cents). There was an increase of 75 per cent in expenditure on industrial research and development in the period 1953–56 from 3,500 million dollars to 6,200 million, and in January 1957, 223,000 scientists and engineers and 396,000 other personnel were engaged in research and development. The Federal Government's contribution rose by 126 per cent to almost 3,000 million dollars and company-financed research and development increased by 45 per cent; in 1956 about 48 per cent of industrial research and development was Government-financed, compared with 37 per cent in 1953. In 1956 basic and applied research projects accounted for nearly one-fourth of the volume of research and development, basic research accounting for 244 million dollars and applied research for 1,200 million dollars. Manufacturing and other industries employed 728,000 scientists and engineers in all activities in January 1957, an increase of 28 per cent on January 1954, as well as 570,000 technicians, of whom 157,000 were employed in research and development. It is estimated that independent commercial laboratories, engineering service firms and trade associations spent more than 100 million dollars on research and development in 1956, and employed a further 5,000 engineers and scientists and 7,000 other personnel on such activities. The chemical industry spent about 55 million dollars on basic research in 1956 and the electrical equipment industry 50 million dollars, aircraft manufacturing coming third with 41 million dollars. Projects in engineering sciences accounted for about 30 per cent of the total cost of basic research and research in chemistry for another 30 per cent, but the most rapid growth in basic research was in the medical sciences, on which the chemical industry spent nearly 25 per cent of its basic research effort.

Haffkine Institute of Bombay

The annual report of the Haffkine Institute for 1958 (pp. iii+56. (Bombay: Government Books Depot; London: High Commissioner for India, 1959.) Rs. 0.62; 1*s.* 1*d.*) records the supply of large

amounts of typhoid vaccine to Ceylon and cholera vaccine to Thailand during epidemics, through the World Health Organization; the demand for cholera vaccine was unusually high, while that for plague vaccine continued to be low. Assistance was also offered in an epidemic of encephalitis, and influenza vaccine was prepared at the Institute and released for distribution. Research activities included investigation of the anti-coagulant action of cobra venom in human and horse blood, and the preparation of new chromogenic substrates for detection and location of enzymes in venom after separation by starch electrophoresis. New thiourea derivatives of the type $R.SO_2.NH.CS.NH.R'$ were synthesized to study their anti-tubercular properties, and tolylbutylamide was prepared by desulphurizing N′-*p*-methylbenzenesulphonyl-N³-*n*-butylthiourea with hydrogen peroxide. Various epidemiological features of salmonellosis are under study and also the pharmacological actions of cholera endotoxins with the view of determining the pathogenicity of the disease, while a gravimetric method has been developed for determining the halogen derivatives of 8-hydroxyquinoline commonly used in medical practice. Manufacture of cholera vaccine by the submerged culture method was attempted, and studies were continued on salivary glands of rabid animals, while trials of influenza vaccine in animals led to the development of a biological test for the standardization of the potency of the vaccine. A study of the iron-binding capacity of serum was started as well as further work on the nutritive evaluation of foodstuffs commonly consumed in the State of Bombay. A list of papers published during the year is included.

Bulletin of the Provisional International Computation Centre

The *Bulletin of the Provisional International Computation Centre*, the eighth issue of which has just appeared, is essentially a serial publication listing computing laboratories, facilities, and conferences throughout the world (Rome: Palazzo degli Uffici, Zona dell'UER, 1960). A few articles are printed from time to time, and the latest issue contains an account of electronic computing facilities in Czechoslovakia. Many of the machines described in this article are unfamiliar in the Western world, and it is to be hoped that fuller details may appear in future issues of the *Bulletin*. It is interesting, also, to read the paper, by Dr. J. E. Holmstrom, on his multilingual terminology project. This seeks to rationalize the computer vocabulary and, most appropriately, takes the new draft British standard on the subject as its basic material.

Comparative Biochemistry and Physiology

The first number of *Comparative Biochemistry and Physiology* (Vol. 1, No. 1 (January 1960). Pp. iv+100. Subscription rate (including postage): (A) For institutions, 20 dollars per volume; (B) for individuals, 15 dollars per volume. New York and London: Pergamon Press, 1960) contains a foreword by Dr. Joseph Needham, in which he points out that in the development of physiology and biochemistry, the emphasis has been on the study of man and the mammals because of the close association of these sciences with medicine. This journal, in which special attention is to be given to the comparative aspects of animal physiology and biochemistry, will help to increase knowledge of all types of animals. It should appeal to zoologists and marine biologists as well as to physiologists and biochemists. The first number contains, among others, communications on toads, fishes, worms, snails and insects. The editors are Dr. G. A. Kerkut, University of Southampton, and Prof. Bradley T. Scheer, University of Oregon, and there is an honorary editorial advisory board of distinguished scientists from many different countries.

Solvents and Dry Cleaning

Unlike laundries, which have been facing serious competition from washing machines and 'launderettes' for more than a decade, the dry cleaning business has been little changed by technical development. During the past twenty years the gross annual turnover of this growing industry has increased from £10,000,000 in 1939 to a probable £35,000,000 to-day. 43,000 people are employed in dry cleaning, two-thirds of them women, in about 2,000 separate companies. The industry handles nearly 400,000 garments a day, which represents about three garments a year for every man, woman and child in Britain. In a dry-cleaning establishment the cleaning liquids are organic solvents, either petroleum-derived white spirit or chlorinated hydrocarbons, such as perchlorethylene. While solvents alone will release and carry away much of the dirt held in the fibres by greasy matter, they make little or no impression on water-soluble sugar and food stains. The advent of the steam gun has speeded up what is called the 'spotting' process. The gun spurts a jet of steam on to the stains, softening them and knocking the food and other particles off the fibres of the garment. In the more modern charged system of cleaning, electronic controls are widely used and give a very high and consistent standard of cleaning. The subject is treated in more detail in *Catalyst* (Vol. 3, winter edition 1959–60).

Flora of the Belgian Congo

The ninth volume of the monumental "Flore du Congo Belge et du Ruanda-Urundi" has now been published by the Institut National pour l'Etude Agronomique du Congo Belge. In its format it follows the same lines as its predecessors. It contains the families Buxaceae, Anacardiaceae, Aquifoliaceae, Celastraceae, Hippocrateaceae, Salvadoraceae, Icacinaceae, Sapindaceae, Melianthaceae, Balsaminaceae, Rhamnaceae, Vitaceae and Leeaceae, some of which are both large and taxonomically complicated. Among the more noteworthy treatments are that of Anacardiaceae by P. Van der Veken, in which the largest genera are *Trichoscypha* with 20 species and *Heeria* with 10; the troublesome Sapindaceae, including 26 species of *Allophylus*, by Hauman; the revision of *Impatiens* by Wilczek and Schulze in which 35 species are enumerated; and the Vitaceae by De Wit and Willems in which the genus *Cissus* runs to no less than 95 species in addition to others imperfectly known. The Hippocrateaceae (which are maintained as distinct from Celastraceae) have been subjected to an elaborate revision by Wilczek. Thirteen genera are recognized, from which it will be seen that the increase in generic segregation in this family, as carried out in recent years by A. C. Smith for America and N. Hallé for Africa, is supported. The largest genus is *Salacia* with 54 species. Students of the family Melianthaceae will have to take notice of a new classification of the formidable genus *Bersama* differing considerably from that of Verdcourt published in 1950.

Health of the Executive

During the past six years the Diagnostic Clinic of the Graduate School of Medicine, University of Pennsylvania, has carried out investigations into the health of 2,000 executives from 70 firms (Medical Affairs, University of Pennsylvania. 1, No. 3, Spring, 1960). On their first examination in the Clinic, 40 per cent of these individuals were found to have previously unrecognized disorders and only 28 per cent were diagnosed as completely healthy; the remaining 31 per cent had had previous illness which currently had an adverse effect on their physical status. Of the newly discovered disease, 57 per cent was considered potentially serious, 34 per cent was minor in importance, and 9 per cent insignificant. Disease in the serious category increased with age. In those 40 years of age or under, only 38 per cent of the newly diagnosed disease was considered serious, while in those over 60 years it rose to nearly 70 per cent. The conditions occurring in the older age groups are as susceptible to treatment as are those in younger individuals. 54 per cent of the newly discovered disease was concentrated in only 13 per cent of the group. There is thus a healthy group of 28 per cent having no disease, either new or old, and 13 per cent of the group having more than half of all the disease found. This small 'disease prone' group is being further examined.

Rugby School Natural History Society

The report of the Rugby School Natural History Society for 1959 contains a number of articles for which the boys concerned were awarded school prizes (Pp. 24+2 plates. Rugby: Rugby School Natural History Society, 1960). One of the most interesting is by I. H. R. Thompson, who obtained a distinguished-work prize. During a period of three years, Thompson has been collecting information about the disposal of dung and carrion and, in this article, describes the part played by beetles in dung and carrion disposal. Although four-footed scavengers and maggots play the greater part in disposal of carrion, Thompson shows that *Necrophori* beetles are responsible for the elimination of considerable quantities of carrion. Almost invariably, the beetles bury the corpses by excavating the earth under it and allowing the body to sink down into the hole. During burial the fur or feathers are usually removed, but the animal is never dissected. Beetles play a much larger part in the disposal of dung than they do of carrion. Although other agents, such as flies and worms, dispose of some dung, the bulk of it is dealt with by beetles. As early as March a patch of dung will yield a quantity of the smaller dung beetles, particularly the smaller *Aphodius*; by May the larger *Geotrupes* can be found in large numbers. About this time the smaller *Aphodius* appear in numbers again.

Population in 1958

The second part of the Registrar General's Statistical Review, which used to be called "Civil Tables", is now published under the title "Tables, Population" (pp. xi+181. London: H.M. Stationery Office, 1960. 11*s.* 6*d.* net). The contents remain unchanged, however; mortality statistics continue to be published in the medical tables, and Part 2 of the volume for 1958 deals with births, marriages and total population. There have been no major changes in the presentation of statistics compared with previous years. Nor were there any striking new trends in the figures themselves during that period. Divorces continued to decline in numbers, new petitions filed stood at the lowest figure since 1945, when the married population was lower in absolute numbers than to-day. Live births were up by about 18,000 compared with the previous years, raising the birth-rate from 16·1 to 16·4 per thousand of the total population and the legitimate birth-rate from 111·5 to 113·9 per thousand married women of reproductive age. Births have been increasing steadily since 1955, and provisional figures for 1958 indicate that the rise has continued in that year.

Principles of the Extraction of Metals

Monograph No. 3 of the series being published by the Royal Institute of Chemistry for the guidance of teachers in schools is concerned with the application of physico-chemical principles to extraction metallurgy (Royal Institute of Chemistry Monographs for Teachers. No. 3: Principles of the Extraction of Metals. By Dr. D. J. G. Ives. Pp. vi+57. London: Royal Institute of Chemistry, 1960. 6*s.*). The author, Dr. D. J. G. Ives, has done a very good bit of work, and no better preparation for a prospective student of metallurgy could well be imagined. The first of the four chapters is concerned with the thermodynamical background, the reader being introduced to, or reminded of, the fundamental concepts. In the next, these are applied to pyrometallurgical processes. Chapter 3 gives a short account of the factors which govern the choice of extraction methods, and the booklet ends with a description of some typical operations such as roasting, the extraction of iron and copper and an outline of hydrometallurgy. This is an excellent introduction to the subject; the only question which can arise is whether the treatment may not be a bit too ambitious for its purpose, but this is a fault, if indeed it be one at all, which is easily remedied.

A Blood Parasite from the California Yellow-legged Frog

During the summer and early autumn of 1958, eleven *Rana b. boyli* were collected from Dutch Mill Creek, Sonoma County, California. Examination of the peripheral blood showed the presence of extracellular and intra-erythrocytic hæmogregarine-like parasites (considered to be *Karyolysus sonomae*) in five of the hosts. Sections and tissue impressions of the viscera indicated that the parasite underwent schizogony in endothelial cells. Donald L. Lehmann reports that this is the first authentic report of a species of this genus to be recorded from any group but the Reptilia (*Proceedings of the American Philosophical Society*, **103**, No. 4; August 1959). Previous reports from the Amphibia have been based upon the morphology of the parasite in the peripheral blood and were not confirmed by demonstrating the vertebrate life-cycle.

The genus *Karyolysus* was established by Labbe (1894) to include those intra-erythrocytic sporozoans capable of fragmenting the nucleus of the host-cell. Reichenow (1921) found that this lytic activity was of uncertain generic worth; he did, however, demonstrate that the parasite could be distinguished from other hæmogregarine-like species on the basis of the life-cycle in the vertebrate host, where schizogony occurs in endothelial cells and the resulting mero-

zoites, following their penetration into erythrocytes, develop into gametocytes. Infections were determined by staining thin smears of peripheral blood with Wright's stain, and more detailed observations were made following Giemsa's stain. Sections of viscera (liver, spleen, kidney, lung and heart) were fixed in alcohol–formalin–acetic acid, stained with Heidenhain's iron hæmatoxylin and cut 15 microns in thickness.

Factors affecting Tubers in *Begonia evansiana* Andr.

This species of *Begonia*, which is evidently a useful plant for investigations of developmental physiology, has buds which consistently form aerial tubers when exposed to short days. These tubers, when given sufficient short-day treatment, do not sprout when afterwards exposed to long days. However, if the plants are transferred to long days after a certain minimal short-day exposure, the tuberizing buds resume growth. There is evidence, from defoliation experiments, that the tuber may itself be sensitive to light (Esashi and Nagao, *Sci. Rep. Tohoku Univ.*, Ser. 4 (Biol.), 24, 81; 1958). These authors (*ibid.*, 25, 191; 1959) have now reported that aerial tubers of *Begonia evansiana* Andr., which are formed under short-day conditions, begin to sprout when isolated and exposed to continuous light while they are actively thickening. After the completion of thickening, however, the tubers sprout no more in response to light. These dormant tubers can be induced to sprout after low-temperature treatments. Whether or not the tubers sprout, they develop roots. In a study of the action of gibberellin on the dormancy of the tubers, Nagao and Mitsui (*ibid.*, 199) found that gibberellin treatments in the concentration range from 0·1 to 100 p.p.m. had no dormancy-breaking effect. Gibberellin treatments at concentrations of 10 and 100 p.p.m. inhibited the sprouting of aerial tubers. The gibberellin effect was greater in more dormant tubers. Gibberellin seemed to induce or prolong the tuber dormancy. The gibberellin-induced dormancy appeared to be broken by low-temperature treatments like natural dormancy. Rooting of aerial tubers was affected but slightly by gibberellin.

Rensselaer Polytechnic Institute

A report on gifts, grants and bequests to Rensselaer, 1958–59, has been issued by the Rensselaer Polytechnic Institute as a supplement to the annual development report, and to emphasize the major importance of such assistance in helping the Institute to achieve its educational goals (pp. 16. Troy, New York: Rensselaer Polytechnic Institute, 1960). Without such contributions the Institute could neither survive nor establish firm plans for future growth. In the past fiscal year such gifts of cash and equipment amounted to 1,362,854 million dollars.

Summer School in Crystal Structure Determination

A Summer School in "Modern Methods of Crystal Structure Determination" will be held in the Manchester College of Science and Technology during August 29–September 9. The main topics to be dealt with, by lectures, practical work and discussions, will be direct methods, Fourier-transform and optical-transform methods, anomalous scattering, and refinement procedures. In addition, there will be some lectures and discussions on recent results in various fields, with assessment of their implications. The fee for the course will be £25, including accommodation in one of the University halls of residence. The fee for attendance without accommodation will be £5. Further information can be obtained from the Director, Extra-Mural Department, The University, Manchester 13.

Association of Consulting Scientists

At the annual general meeting of the Association of Consulting Scientists, held in London recently, the following officers and Council for 1960–61 were elected: *chairman*, Dr. J. G. Davis; *hon. treasurer*, Dr. G. W. Ferguson; *hon. secretary*, Mr. W. H. Stevens; *other members*, Dr. M. Barent, Dr. H. H. Chambers, Dr. Julius Grant, Dr. J. H. Oliver, Mr. O. W. Roskill, Mr. F. G. Sarel Whitfield.

The Linnean Society of London

At the anniversary meeting of the Linnean Society of London, held on May 24, the following were elected officers and Council for the session 1960–61: *president*, Prof. C. F. A. Pantin; *treasurer*, The Earl of Cranbrook; *secretaries*, Dr. C. R. Metcalfe (botany), Dr. H. G. Vevers (zoology), Dr. John Smart (editorial). *Vice-presidents* appointed were: Mr. E. J. H. Corner, The Earl of Cranbrook, Asst. Prof. H. R. Hewer and Dr. W. G. Templeman. *New members of Council*: Dr. A. J. Cain, Prof. A. R. Clapham, Dr. W. P. Croweroft, Mr. F. C. Deighton and Mr. J. F. Levy. *Linnean Gold Medals* were presented to: Dr. Libbie Henrietta Hyman and Dr. H. Hamshaw Thomas. *The Trail Award and Medal* was presented to Dr. Laurence Ernest Rowland Picken. Prof. Dr. C. G. G. van Steenis and Dr. Robert E. Snodgrass were elected foreign members and Mr. H. F. Comber, Mr. Dennis A. Kempson and Prof. Alexander Eric Kohts were elected associates *honoris causa*.

Royal Society of South Africa

The following were elected as officers and Council for 1960: *president*, Dr. S. M. Naudé; *honorary general secretary*, Mr. W. J. Talbot; *honorary treasurer*, Dr. N. Sapeika; *acting honorary editor of transactions*, Mr. A. M. Talbot; *honorary librarian*, Dr. J. H. Day; *other members of council*, Dr. R. A. Alexander, Dr. S. F. Bush, Dr. A. L. Hales, Dr. W. E. Isaac, Dr. M. R. Levyns, Dr. R. H. Marloth, Dr. S. H. Skaife, Dr. R. H. Stoy and Dr. C. L. Wicht.

The Leopoldina German Academy of Sciences: Elections

Die Deutsche Akademie der Naturforscher, Halle, recently elected the following new members to the Sections indicated: *Astronomy*, Prof. Paul ten Bruggencate (Göttingen), Prof. A. A. Michailow (Pulkowo, Leningrad), and Prof. Martin Schwarzschild (Princeton); *Medicine*, Prof. Heinrich von Hayek (Vienna), Prof. Josef Kimmig (Hamburg), Prof. Friedrich Matzen (Leipzig), Prof. Hans Netter (Kiel), Prof. Karlheinz Sommer (Halle), and Prof. Werner Wachsmuth (Würzburg).

"Ataxia in Hens poisoned by Tri*para*ethylphenyl Phosphate"

With reference to her communication under this title in *Nature* of January 23, p. 247, Miss Ann Silver writes: "I should like to emphasize that the first

evidence that this substance produces ataxia in hens was observed by Bondy, Field, Worden and Hughes at the Nutritional Research Station in Huntingdon, and it was first synthesized in the laboratories of Coalite and Chemical Products, Ltd., and the sample tested by me was provided by Dr. H. F. Bondy".

University News :

Belfast

THE following appointments to lectureships are announced : Dr. S. M. Nelson (organic chemistry) ; Dr. A. R. Johnston (physics) ; Dr. L. T. Threadgold (zoology) ; Mr. T. J. Black and Mr. D. McCloy (mechanical engineering).

Birmingham

THE title of reader in reactor physics has been conferred on Dr. J. Walker, senior lecturer in physics. Appointments to lectureships are as follows : Gillian Vaisey (civil engineering) ; G. Lees (road materials) ; Dr. G. H. Burkhardt (mathematical physics) ; Dr. M. J. Lawrence (genetics) ; G. H. Trengrouse and E. Amini (mechanical engineering) ; Dr. D. C. Champeney and D. A. O'Connor (physics). D. R. Milner has been appointed Lucas Fellow in metal-joining in the Department of Industrial Metallurgy for seven years.

Edinburgh

THE following have been appointed Imperial Chemical Research Industries Fellows : Dr. J. L. Holmes, Dr. K. R. Jennings, Dr. G. R. H. Jones, Dr. A. J. Charlson, and Dr. J. W. Sutherland. Mr. M. J. O'Hara has been appointed lecturer in geology and Mr. E. R. S. Fifoot, University librarian (as from October 1).

Leeds

APPOINTMENTS to lectureships are as follows : W. G. Collins and J. A. Fox (civil engineering) ; Dr. C. M. Reeves (electronic computing) ; C. G. Woods (pathology) ; Dr. P. J. Wyllie (experimental petrology). Dr. D. M. Robertson has been awarded the Portsmouth fellowship for research in multiple sclerosis for three years. The Department of Zoology has received a grant of £2,055 from the Department of Scientific and Industrial Research for research projects under the direction of Dr. Broadhead. The Department of Biomolecular Structure received grants of 152,517 dollars from the United States Public Health Authority and £1,100 from the Medical Research Council ; the latter is to be used in establishing a research fellowship. The Houldsworth School of Applied Science has received among other grants £3,500 from the British Coke Research Association and £1,200 a year to establish a research fellowship in ceramics from the Thermal Syndicate, Ltd.

Nottingham

DR. CYRIL CLIFFORD ADDISON, at present reader in the Department of Chemistry, has been appointed professor of inorganic chemistry as from October 1. Dr. Addison graduated at Durham in 1934 and obtained the research degrees of that University of Ph.D. in 1937 and of D.Sc. in 1947. During the War he worked for the Ministry of Supply at Runcorn and Preston, and was later head of the Analytical Research Laboratories at Sutton Oak. He was appointed lecturer in inorganic chemistry at Nottingham in 1946, and in 1952 the title of reader was conferred upon him.

Dr. Maurice William Partridge, at present Lord Trent reader in pharmaceutical chemistry, has been appointed to the Lord Trent chair in the same subject as from October 1. Dr. Partridge graduated with honours at University College, Nottingham, and in 1941 was awarded the research degree of Ph.D. of the University of London. During the War he was research assistant to the British Pharmacopœia Commission and later was senior scientist in the chemical research laboratories of Boots Pure Drug Co., Ltd. He was appointed lecturer in pharmaceutical chemistry in 1947, and in 1955 was appointed to his present readership.

Oxford

THE following appointments to readerships have been announced : Dr. E. P. Abraham (chemical pathology) ; Dr. E. Bülbring (pharmacology) ; Dr. W. A. Waters (physical organic chemistry) ; and Dr. N. Kurti (physics).

St. Andrews

THE following appointments to senior lectureships are announced : Dr. Ann R. Sanderson, Department of Zoology, Queen's College ; and Dr. D. V. Osborne, Department of Natural Philosophy, St. Salvator's College.

Announcements

LORD HIVES, who was the first chairman of the National Council for Technological Awards, has been given an honorary diploma in technology (engineering) ; this is the first honorary award to be made by the Council.

THE Chemical Research Committee of the Indian Council of Scientific and Industrial Research is organizing a two-day symposium on "Proteins" at Mysore during the first week of August. Further information can be obtained from Dr. A. Sreenivasan, Central Food Technological Research Institute, V. V. Mohalla, P.O., Mysore 2.

THE tenth International Congress of the History of Science will be held in the United States during August 26–September 2, 1962. Opening sessions of the Congress will be held at Cornell University, Ithaca, New York, and the concluding sessions will be held at the American Philosophical Society, Philadelphia, Pennsylvania. The president of the Congress is Prof. Henry Guerlac, of Cornell University. Further information can be obtained from the secretary, Prof. C. Doris Hellman, Cornell University, Ithaca, New York.

THE fourteenth congress of the International Scientific Film Association will be held in Prague during September 16–24, and will include, as in previous years, a festival of films on "Presenting Science". Responsibility for selecting the British film entry for the Congress rests with the Scientific Film Association of Great Britain. Further information can be obtained from the Scientific Film Association, 3 Belgrave Square, London, S.W.1.

AN international conference on "Natural Rubber Research", sponsored by the Rubber Research Institute of Malaya with the support of the Government of the Federation of Malaya and the Rubber Producers' Council, will take place in Kuala Lumpur during September 26–October 1. Further information can be obtained from the Rubber Research Institute of Malaya, P.O. Box 150, Kuala Lumpur.

NEW PHYSICS LABORATORY OF THE UNIVERSITY OF READING

THE new physics laboratory of the University of Reading was opened on May 17 by Sir George Thomson, who named the building "The J. J. Thomson Physical Laboratory". On the occasion of the opening, honorary degrees were conferred on Dr. T. E. Allibone, Prof. N. F. Mott, Prof. F. Seitz and Sir George Thomson. It is of interest to recall that Sir J. J. Thomson received an honorary degree from the University in 1935 and that both the first professor of physics at the University of Reading (J. A. Crowther) and the present professor (R. W. Ditchburn) worked in the Cavendish Laboratory under him.

The Laboratory was planned to accommodate undergraduate classes of up to 100 in each of the first two years (post-intermediate), and about thirty in the special honours final year. Facilities for about fifty research workers (including members of staff) were also provided. It was expected that the intermediate science class would be very small, but that teaching at this level would be required for about 100 students in agriculture, etc. It now appears probable that the numbers of students in these elementary classes will be much smaller than was expected, but the teaching laboratory space released will be required to deal with the large increase of students now predicted for the next decade.

The Laboratory is a three-storey steel-framed building with a floor area of about 63,000 sq. ft. The structure has brown-grey brick walls and the greater part has a low pitch roof covered in copper. The western end of the Laboratory, connected to the main building by a two-storey glazed link, is the lecture theatre block. This has a flat roof surrounded by a parapet wall. The ground floor contains two lecture theatres, with a projection room for each and a preparation room common to both. On the first floor, above the lecture theatres, are two large teaching laboratories for the general honours students and for special honours students in their first and second years. These laboratories cover an area of 5,000 sq. ft. with a single-span roof, internal steel partitions being used to provide dark-rooms, etc. The absence of internal structural pillars and load-

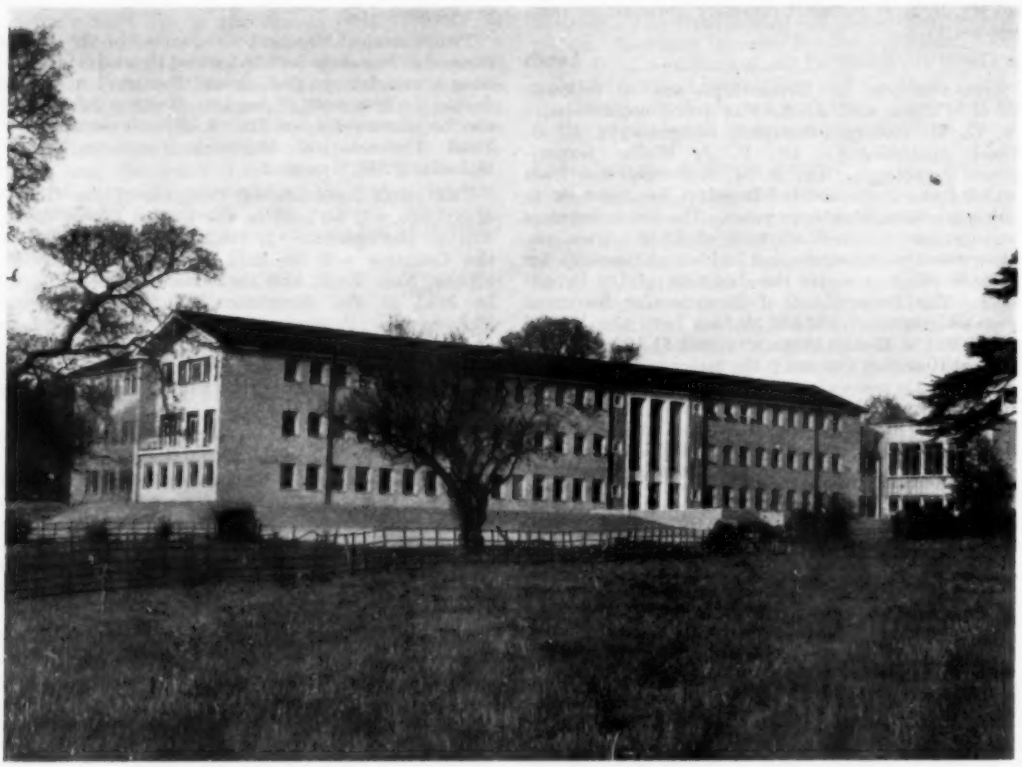

Fig. 1. The J. J. Thomson Physical Laboratory, University of Reading

bearing walls enables the space to be utilized in a very economical way.

The larger lecture theatre contains seating for 180 and the smaller for 105. Each has bench-type seating, specially modelled for maximum comfort, and fitted with hinged writing flaps. In each theatre the lecturer's demonstration bench is equipped with all laboratory services and, in addition, the electrically operated windows, blinds and dimming apparatus for the lighting can be controlled from it. The walls behind the demonstration benches are prepared to form a permanent projection surface 12 ft. wide and 6 ft. high, on which two pictures can be projected side by side. Below the projection surface is a 12-ft. glass chalk-board. On the left-hand side of the central wall is a splayed wall with a translucent glass chalk-board, which can be illuminated from behind when the room is darkened; on the right-hand side is another splayed wall fitted with a chalk-board of conventional type.

Heating in the lecture theatres is by means of radiant wall panels combined with a heated air plenum ventilation system. Elsewhere in the building heating is by 'Frenger' acoustic heated ceilings. All windows are double-glazed to reduce heat losses. Many of the rooms are provided with inlet and extract ventilation, particularly those fitted with dark blinds.

The main laboratory is planned on a repeating bay unit 7 ft. 6 in. wide and 16 ft. deep, with laboratory services arranged beneath the windows in a service rack. The services available in every bay of the building include alternating current supplies at 415 V. (3-phase) and 240 V. and 6 V. (single phase) and direct current at 240 V. and 24 V. Hot and cold water, gas and compressed air are also available.

It was estimated that one and a half million gallons of cooling water per annum would be required for diffusion pumps, compressors, etc., and a recirculation system is therefore provided. This service is generally available in the centres of rooms, together with 240 V. a.c. and one other service at choice.

Walls throughout the building are plastered and tinted and the doors are decorated in bright colours. The floors of most rooms are covered with 'Tarkett P.V.C.' tiles and the corridors with 'Korkoid'. The library has been designed to enable the beautiful surroundings of the Laboratory to be seen to advantage. Part of a gift from Imperial Chemical Industries, Ltd., has been used to provide books for the library, which will be especially useful during the next three years when the University Library is still on the old University site. Office accommodation for a theoretical physics group is provided near the library. This group is working on the application of quantum mechanics and statistical mechanics to problems in solid state physics, in particular crystal lattice vibrations, lattice imperfections and magneto-optic effects in semiconductors.

Most of the experimental work in the Laboratory is concerned with the physics of the solid state. One research group is studying the effects of imperfections in crystal structure on physical properties, particularly with reference to radiation damage (defects produced by irradiation with pile neutrons and other high-energy particles). A 2-MeV. Van de Graaff generator (purchased with a grant from the Department of Scientific and Industrial Research) is used to produce defects in a controlled way. It is housed in a small building at a convenient distance from the main laboratory.

A section of the research space is assigned to the radio-tracer laboratory, which is mainly concerned with atomic diffusion in metals. This laboratory contains also a mass spectrometer which is used to assist biological as well as physical research.

A research group, supported by the Admiralty, is working on the surface properties of semiconductors. Methods of breaking germanium in a very high vacuum (10^{-8}–10^{-10} mm. mercury) so as to expose a crystal plane have been devised, and the gradual contamination of these surfaces is being studied. Work on the etching of surfaces is being studied using high-quality optical microscopes, and an *E.M.*6 electron microscope is on order. Associated Electrical Industries, Ltd., have kindly agreed to provide a technical assistant to work this instrument for three years. Studies of solids by X-ray and electron diffraction are also in progress. The Royal Society has given a grant to assist the development of high-voltage electron diffraction using the Van de Graaff generator.

The Laboratory is equipped for spectroscopic studies for the infra-red, visible, ultra-violet and soft X-ray regions. The apparatus is used for measurements on solids chiefly in the infra-red and visible regions and in the vacuum ultra-violet. The results on gases have applications to studies on the upper atmosphere and to the physics of hot plasmas.

A biophysical investigation on vision is supported by the U.S. Public Health Service.

When the Laboratory was designed, the number of students in the University of Reading was expected to become stable at a figure in the range 1,500–2,000. An increase to 3,000 is now contemplated. The Laboratory will have to provide for larger numbers in the Physics Department and also for the early development of at least two new departments in the physical sciences. The design of the building provided for possible extension which, it was thought, might be needed after twenty-five years. The new conditions have led to discussion of this extension even before the building was opened.

R. W. Ditchburn

GOVERNING TECHNICAL COLLEGES

A HANDBOOK, "The Technical Colleges and their Government", issued by the Joint Federation of British Industries Technical Colleges Committee under the auspices of the Federation's Education Committee, provides a concise but keen industrial appraisal of the structure of technical education, its historical background, academic pattern and finance as well as a close discussion of the government of technical colleges (pp. vii+44. F.B.I. Handbook. London: Federation of British Industries, 1960. 5*s*.).

Appendixes included a policy statement on the finance of advanced sandwich courses issued by the Federation, the Ministry of Education's Circular 7/59 on Governing Bodies for Major Establishments, and a selected bibliography which is thoroughly representative.

The recommendations based on this survey emphasize that it is the function of governors to ensure that their college fulfils its role as an academic institution within the national and regional framework of further education. To do so involves strong links with industry, commerce and the professions; a constant awareness of the changing needs of their students; and adequate powers to implement the governing body's decisions. Governors nominated by special interests can and should bring their special experience or background to bear on the affairs of the college, but the handbook emphasizes that the governors serve to promote the interests of the college and that they should be aware that each college occupies a distinct place in the pattern of further education as a whole.

On size, the handbook suggests a membership of about twenty as ideal, and it should include members of the professions with which the college is mainly concerned and of the universities. The contribution which headmasters of secondary schools could make should be considered and provision for co-opting independent persons with special knowledge or experience, representative of ex-students and for continuity is recommended. Stress is laid on the importance of the full implications of the recommendations of the Ministry of Education in regard to the powers of the governing body, and the handbook stresses the importance both of the power of governors being commensurate with the responsibilities they are asked to assume and of adjusting procedure to ensure the best possible use of the governors' time.

One meeting of two to three hours a term is suggested as covering most requirements, but apart from attendance at formal meetings of the governing body and its committees, governors can and should play a valuable part in the life of the college, and it is recommended that they should be encouraged to do so. Departmental advisory committees are usually appointed for each major discipline studied at a college, and these should consist of experts from industry or commerce, members of the governing body and the teaching staff and representatives of the appropriate professional body. They may also include H.M. Inspectors and should have room for co-opted independent experts. Close links between the advisory committees and the governing body are essential, but the constitutional position of advisory committees will vary according to the subject, and in fields which are undergoing continuous change, the establishment of permanent advisory committees may be advisable.

SOUTH-EASTERN UNION OF SCIENTIFIC SOCIETIES

THE South-Eastern Union of Scientific Societies held its sixty-fifth annual congress in Ipswich, during April 22–25, under the presidency of the Earl of Cranbrook. The headquarters of the congress was at Ipswich School, where an exhibition of natural history was on display. A young naturalists' evening was arranged and questions from the audience were answered by a panel under the chairmanship of Maxwell Knight. Lord Cranbrook's presidential address to the Union was on "East Anglian Mammals", and he began by explaining that most of the area is highly cultivated, therefore the habitats are largely man-made. The red deer in East Anglia are possibly the progeny of escapes of those introduced from Scotland and are larger than those of the same species in Scotland, the increased size being due to an environmental change. Introduced roe deer are now flourishing in the Breckland.

Dealing with the seal population he said that both common and grey species occurred, a colony of grey seals being established during 1958 on Grove Sands, off Yarmouth, which are dry at the high-water mark. Ringing experiments are being made for recording movements of individuals, breeding places and the months on which the young of different species are born.

Lord Cranbrook said that for some obscure reason there appeared to be a natural barrier on the Essex-Suffolk border which prevented a number of mammals from crossing to suitable habitats in Norfolk and Suffolk. The distribution of the dormouse, horse-shoe bat and grey squirrel is limited to the south of the Stour valley although the red squirrel is abundant in the coniferous woods of the Breckland. Coypu numbers increase and the animal has become a nuisance, damaging farm crops and banks of ditches and streams. Scientific investigation is necessary to find the steps to be taken for control. Defending the policy of the Forestry Commissioners in planting mainly conifers, he said that pine forests are useful for the preservation of mammals, badgers are increasing in numbers, and the pine marten, which is increasing its range, may be expected to reach the area in the near future. The question of the black rat race in the Fens needs careful consideration; there appears to be two varieties common in the south and east of England and their control may be necessary. He concluded his address by comparing the growth of teeth of the field and water voles. In the former the teeth continue to grow throughout their life, as much as 2½ in. a year, a corresponding amount being worn away by the hard food they consume. In the case of the water vole the teeth cease to grow when the animal is adult, calification then aiding the resistance to wear.

The presidential address to the Archæological Section was given by Mr. N. Smedley on "Archæology in Suffolk". Speaking on the early investigators he mentioned the finding of an Acheulean implement by John Frere in 1797 and the scepticism with which the suggestion of man's existence before the present world conditions was received. He traced the development of the beaker pottery forms through the neolithic, bronze and iron ages. Roman times in Suffolk

showed a specialized form of kiln with no raised floor during firing. Mr. Smedley had supervised the reconstruction of a kiln of this type to prove the possibility of obtaining the necessary draught and temperature for the baking of clay vessels.

Mr. F. W. Simpson gave the presidential address to the Botanical Section, his subject being "Changes in the Suffolk Flora during the Past 100 Years". He emphasized the need for the conservation of habitats, pointing out that since Henslow's Flora was published in 1860 about 75 natives had become extinct, another 40 will soon be gone and others are in danger of such a fate. This is largely due to suitable habitats being reduced by human development for building purposes. The introduction of aliens may increase the number of species, and for an understanding of the general situation it is important that records of first and last appearances of species should be kept.

An appointment in America prevented Dr. R. G. West from delivering his presidential address to the Geological Section in person, but a recording was available on "Investigating Glacial and Interglacial Geology in East Anglia". Three methods have been used in the determination of the relations of the deposits: tracing the origins of the boulders by the identification of the materials; the study of the orientation of the boulders by the alignment of the long axes in undisturbed deposits; pollen analysis, the size and pattern of the pollen showing the genera and sometimes the species present, so from the vegetational sequence a climatic one could be deduced. Four main glacial periods may be correlated by these methods, the oldest Cromerian, followed in turn by Lowestoft, Gipping and Hunstanton. Implements occurs throughout, the most prolific deposit being the Hoxnian, between the Lowestoft and Gipping glaciations, where Acheulean specimens occur in a deposited brick-earth in conditions at least as warm as at the present time, the pollen grains including pine, birch and oak.

Mr. S. Beaufoy chose for his presidential address to the Zoological Section the subject of "Some East Anglian Butterflies". Illustrating his address with coloured slides of outstanding merit, he commenced by confirming the opinion that a natural boundary appeared to prevent species, including the Speckled Wood and Chequered Skipper butterflies, from occurring in Suffolk although recorded in adjacent Essex. The life-history of the Swallowtail was described, a British sub-species occurring in Norfolk, the caterpillars feeding on wild carrot and, also, readily taking the leaves of the cultivated variety. The Silverwashed Fritillary is fairly common in woodlands, the eggs being deposited on moss during July and August, the caterpillar's first meal being its egg shell. It then hibernates and nothing more is eaten until the following spring when, by feeding on violet leaves, it completes its larval stage. The White Admiral, quite common in parts of East Anglia, deposits its eggs on honeysuckle, the caterpillars feed for about three weeks and then hibernate throughout the winter. The Purple Emperor was last recorded in 1919, the Large Tortoiseshell has become rare and an attempt to reintroduce the Large Copper has not been entirely successful.

Mr. J. E. Marston, the local secretary responsible for the arrangements of the congress, gave an illustrated talk on "Suffolk Ponds and Pond Animals". Tracing the life history of the mayfly he said that, although the life of the adult did not exceed 24 hr., the nymph takes three years to develop to this stage. The speaker wished for a full investigation into the breeding habits of the freshwater shrimp; preliminary researches at Belstead House have yielded some unexpected results.

Favourable weather conditions enabled the full programme of excursions to be carried out. The archæologists visited Little Wenham Hall, Earl Stonham church and Helmingham Hall. As a prelude to Mr. Smedley's lecture a visit was made to Christchurch Mansion under his direction. The botanists made an excursion to study the early spring flora of the heaths of East Suffolk and the coast, at Woodbridge, Shingle Street, Butley and Chillesford. The geologists were conducted around the quarries at Great Blakenham and Creeting St. Mary to view sections revealing the effect of moving ice-sheets during the Glacial periods.

The general excursion was to Flatford Mill Field Study Centre, where the warden, Mr. F. J. Bingley, conducted the party around the house and grounds, explaining the aims and work of the Field Study Council.

The sixty-sixth annual congress of the Union will be held next year, during May, at Haslemere.

F. J. Epps

ACTINOMYCIN

DURING March 31–April 1 a conference was held, under the auspices of the New York Academy of Sciences, on "The Actinomycins and Their Importance in the Treatment of Tumours in Animals and Man". The conference consisted of three sessions concerned with: "Microbiological and Chemical Aspects", "Pharmacology and Experimental", and "Clinical". Prof. Selman A. Waksman was the general chairman of the sessions.

Actinomycin was discovered in 1940 in the laboratories of the Soil Microbiology Division of the New Jersey Agricultural Experiment Station, Rutgers University. It was isolated from a culture of an actinomycete, later described as *Streptomyces antibioticus*. Since then, numerous other forms of actinomycin have been isolated throughout the world. The first was designated as A, and the subsequent ones as B, C, D, X, etc. Each of these consists of several chemical entities. Nearly fifty letters, numbers and subscripts have been used to designate the various entities. To clarify the nomenclatural problem, it has been suggested that names be applied to the various preparations only after the chemical constitution of each new form has been elucidated.

The actinomycins are produced by a variety of different species of *Streptomyces*. They represent a group of chromopeptide antibiotics which differ only at one site in the peptide portion of the molecule. The nitrogen source supplied to the medium influences greatly the quantitative and qualitative composition of the actinomycins. The chemical composition of the actinomycins can be controlled during bio-

synthesis, notably through the addition of sarcosine, pipecolic acid, valine, *iso*leucine, and certain other amino-acids to the medium. The chemistry of the actinomycins was reviewed by Prof. A. W. Johnson, of the University of Nottingham, and Prof. Hans Brockmann, of the University of Göttingen.

All the actinomycins contain the same chromophore group, which has been identified as 2-amino-4 : 6-dimethyl-3-phenoxazinone-1 : 9-dicarboxylic acid. The structural differences among the various actinomycins make for differences in their antimicrobial, cytostatic and toxic properties; but there appears to be limited differences in their therapeutic indices against animal tumours and lymphogranulomatosis. An attempt was therefore made to obtain actinomycin derivatives with better therapeutic indices, by means of chemical alterations in the chromophore or peptide portions of the actinomycins. Structural changes in the actinomycin chromophore may be accomplished if one exchanges the amino-grouping of the chromophore for chlorine, and if the resulting chloro-actinomycin is then made to react with primary or secondary amines, or with other compounds which contain amino or imino groups. Brockmann reported total synthesis of actinomycin C_3, identical in chemical and biological behaviour with the natural preparations.

Among the various actinomycin preparations tested upon animals and in clinical medicine, C (the German preparation) and D (the American preparation) received the greatest attention at the conference. The Sloan-Kettering group reported that fatal intoxication with actinomycin D, following supralethal doses which caused death in less than 24 hr., might possibly be due to extensive damage of the intestinal epithelium allowing entrance of an endotoxin or a toxic polysaccharide into the blood stream. The antibiotic, at the LD_{50} dose, induces extreme hypoplasia in the bone marrow. Neutrophilic granulocytosis is the usual finding; the short survival period is probably insufficient time to reflect the full consequence of destruction of bone marrow. Changes in the lymphoid tissues are presumably direct actions of actinomycin D, since they can be observed in adrenalectomized as well as in normal rats. Actinomycins D and F_1 produce about the same increase in survival time (less than 100 per cent) when tested against a variety of leukæmias. Actinomycin F_1 is active against spontaneous mammary cancer of mice, whereas actinomycin D is without effect. The majority of the 32 actinomycin derivatives tested have little effect against the leukæmias.

A group from the Institute of Microbiology of Rutgers University reported that the antitumour effects of actinomycins II, III and IV have been studied in four ascitic tumour systems. The intraperitoneal therapeutic index of actinomycin II was found to be twice that of actinomycin IV and 4-fold that of actinomycin III in the Gardner lymphosarcoma system. The effective dose chosen for this calculation was the minimal amount producing a 100 per cent increase in survival time. The maximal effect of the actinomycins was attained with a total dose ranging from 1/15 to 1/40 of the LD_{50} dose, when the actinomycins were administered intraperitoneally for three successive days. The group from the Children's Hospital (Boston) reported that the most striking effects on transplanted tumours in mice were produced on the *S*91 melanoma, *P* 1534 lymphatic leukæmia, *DBRF* mammary adenocarcinoma, and the Ridgway osteogenic sarcoma. A synergistic effect with actinomycin D and radiotherapy has been demonstrated in mice bearing the Ridgway osteogenic sarcoma, as well as on normal mouse skin. A variety of heterologous tumours, including human cancers, have been grown in hamsters and afterwards treated successfully with actinomycin D.

The work of the Bayer group in Germany with actinomycin C and other preparations were reported by Dr. C. Hackmann. In tissue-culture studies using HeLa cells, morphological changes of the nucleoli occur which are characteristic of the actinomycins. These changes have not been observed so far with other antitumour agents.

Clinically, when used alone or in conjunction with radiation, actinomycin D has produced objective remission of varying duration in a limited number of cancers. Regression of tumour-size as well as disappearance of metastatic lesions (especially pulmonary) may last for a few weeks to several years (up to five). The tumours which have thus far been found sensitive to actinomycin D are: Wilms's tumour, rhabdomyosarcoma, Ewing's sarcoma, chorioncarcinoma of testis and uterus, lymphomas (Hodgkin's disease and lymphosarcoma), and bronchogenic carcinoma. Of these 'responsive' tumours, the most extensively studied has been Wilms's tumour. The greatest proportion of regressions occurred in this group of patients. With the other tumours considered to be sensitive, a smaller number showed adequate results. Responses in children are more favourable than in adults.

Actinomycin is capable of reactivating, in distribution and amount, the skin and mucous membrane reactions produced by initial irradiation. When used during the course of irradiation, actinomycin can also cause severe reactions. This potentiating effect of actinomycin D must be considered when combination therapy is contemplated. Doses of X-ray and actinomycin D must be adjusted and scrupulous care taken to exclude all normal tissues possible from the radiation beam. Most, if not all, of the patients treated with actinomycin show one or more toxic manifestations, all of which are reversible when the drug is discontinued. The severity of toxicity varies markedly, being only partly dependent upon the dose of drug. Toxicity usually does not become apparent until 2–4 days after the course of actinomycin D is stopped, and may not be maximal before 1–2 weeks have elapsed. Nausea and vomiting, however, occur within several hours after administration.

Actinomycin C has an almost selective cytostatic effect on lymphatic tissue. Its clinical use is limited therefore to Hodgkin's disease and chronic lymphatic leukæmia. In Hodgkin's disease, Begemann reported that remissions occurred in 22 of 54 patients whose disease was far advanced and refractory to other treatment, and in 19 of 38 patients who were responsive to other forms of therapy (in whom actinomycin was used in combination with X-rays and other drugs). Remissions, however, are short-lived. Because actinomycin C does not produce depression of the bone marrow, it is particularly suited for therapeutic trials in patients with aleukæmic forms of Hodgkin's disease or chronic lymphatic leukæmia. Although bone-marrow depression does not occur, actinomycin C also produces gastrointestinal, dermatological, neurological, and soft tissue toxicity including: nausea, vomiting, diarrhœa, loss of feeling of well-being, gingivitis, stomatitis, glossitis, alopecia, polyneuritis, and irritation of veins and thrombophlebitis.

U.S NATIONAL ACADEMY OF SCIENCES

ELECTIONS TO MEMBERSHIP

AT the recent annual meeting of the U.S. National Academy of Sciences, Dr. Lloyd V. Berkner, president of Associated Universities, Inc., in New York City, was elected to a four-year term as treasurer of the Academy. Prof. G. Evelyn Hutchinson, Sterling professor of zoology, Yale University; and Dr. Robley C. Williams, professor of virology and research biophysicist, University of California, were elected members of the Council of the Academy in succession to Dr. Frederick Seitz and Dr. Harry L. Shapiro.

Also at this meeting, thirty-five new members were elected as follows:

Prof. H. L. Anderson, director of the Enrico Fermi Institute for Nuclear Studies;

Dr. A. V. Astin, director of the U.S. National Bureau of Standards since 1952;

Prof. N. Bloembergen, professor of applied physics, Harvard University;

Prof. A. T. Blomquist, professor of organic chemistry, Cornell University;

Prof. H. G. Booker, professor of electrical engineering, Cornell University;

Prof. A. C. Braun, professor of bacteriology, Rockefeller Institute;

Prof. O. Chamberlain, professor of physics, University of California;

Prof. N. R. Davidson, professor of chemistry, California Institute of Technology;

Prof. W. Feller, Higgins professor of mathematics, Princeton University;

Dr. Herbert Friedman, superintendent, Atmosphere and Astrophysics Division, U.S. Naval Research Laboratory.

Dr. R. Galambos, chief of the Department of Neurophysiology, Walter Reed Army Institute of Research;

Prof. M. Gell-Mann, professor of theoretical physics, California Institute of Technology;

Prof. D. R. Griffin, professor of zoology, Harvard University;

Prof. H. S. Gutowsky, professor of physical chemistry, University of Illinois;

Prof. B. Haurwitz, professor of astro-geophysics, University of Colorado;

Prof. H. D. Hedberg, professor of geology, Princeton University;

Prof. K. F. Herzfeld, professor of physics and head of department, Catholic University of America, Washington, D.C.;

Prof. C. I. Hovland, Sterling professor of psychology, Yale University;

Dr. R. J. Huebner, chief of the Laboratory of Infectious Diseases, U.S. National Institute of Allergy and Infectious Diseases;

Dr. A. B. Kinzel, vice-president in charge of research, Union Carbide and Carbon Corporation;

Prof. S. E. Luria, professor of microbiology, and chairman, Microbiology Committee, Massachusetts Institute of Technology;

Prof. D. Mazia, professor of zoology, University of California at Berkeley;

Prof. S. Moore, professor of biochemistry, Rockefeller Institute;

Prof. T. T. Puck, professor of biophysics and head of department, University of Colorado Medical School;

Prof. R. W. Sperry, Hixon professor of psychobiology, California Institute of Technology;

Prof. W. H. Stein, professor of biochemistry, Rockefeller Institute;

Prof. W. S. Stone, professor of zoology and director of gene research, University of Texas;

Prof. G. J. Stork, professor of chemistry, Columbia University;

Dr. R. N. Tousey, head of the Rocket Spectroscopy Branch, Atmosphere and Astrophysics Division, U.S. Naval Research Laboratory;

Prof. J. B. Wiesner, director, Research Laboratory of Electronics, Massachusetts Institute of Technology;

Prof. G. R. Willey, Bowditch professor of Central American and Mexican archæology, Harvard University;

Prof. C. M. Williams, professor and chairman, Department of Biology, Harvard University;

Dr. O. C. Wilson, astronomer, Mount Wilson and Palomar Observatories;

Prof. C. N. Woolsey, Charles Sumner Slichter research professor of neurophysiology, University of Wisconsin;

Prof. A. Zygmund, professor of mathematics, University of Chicago.

'EIDOPHOR' TELEVISION TECHNIQUE

A DEMONSTRATION of the 'Eidophor' system of projection television was given during the week beginning May 2 by Messrs. Ciba Clayton, Ltd., at Belle Vue, Manchester. A studio was set up in the technical service laboratories, and the programme was transmitted 'live' a distance of 1½ miles to Belle Vue by micro-wave link. The aim was to demonstrate the use of the new system to enable lecture demonstrations to be given to very large audiences. The organizers are to be congratulated; it was a most impressive demonstration.

The essential feature of the 'Eidophor' system is a special cathode-ray tube which modulates a beam of light derived from a high-power xenon arc. In principle, therefore, the limit to the size of screen used and the brightness of the picture is set only by the light output of the lamp, and not, as in most other systems, by the characteristics of the phosphor of a projection tube. A screen of up to about 800 sq. ft. may be used for black-and-white projection with the system in its present state of development; there is necessarily some diminution in brightness in colour television, but even then screens up to about 200 sq. ft. may be used.

It is not proposed to describe in detail the technical aspects of the system except to comment first on its

extreme ingenuity, and secondly, on the skill and patience which must obviously have been used in its perfection. The 'modulator' is a mirror over which a thin film of oil is continuously re-formed. The thickness of the oil film is controlled by the electric charge pattern on its surface, and this in turn is controlled by the cathode-ray beam. The variations in thickness are used to control the intensity of the light from the source. The idea is simple in conception, but there are considerable technical difficulties that have had to be surmounted. The 'Eidophor' projector may be fed by signals from a broadcasting network or from a closed-circuit link. The large amount of light available makes possible the use of a simple frame-sequential colour system, and the demonstration at Belle Vue was of this kind. An ordinary television camera is used to provide the signals, but a three-colour rotating filter is mounted in front of the lens. A similar rotating filter—synchronized by special signals—rotates in front of the light source in the projector. The rate of frame repetition is 75 per sec., so that a complete set of three coloured images is transmitted in the normal frame-time of one-twenty-fifth of a second. The colour quality—though not perfect—was quite adequate for technical demonstrations.

A series of short lectures was given, starting with a technical description of the 'Eidophor' by Arthur Garratt and followed by three or four demonstration lectures of dyeing processes and techniques, including paper chromatography. After the lectures the audience was invited to ask questions, and these were answered from the studio over the television link. It was this last feature that demonstrated most clearly the fascinating possibilities of large-screen television. As research becomes more and more concerned with complex apparatus, so lectures with demonstrations become more and more difficult except for very small audiences. A really good colour projector would make it possible for a large audience to see and hear lectures with the same comfort as a single person; it is possible, of course, to use close-up techniques and even to transmit the images from microscopes and other optical instruments. The 'Eidophor' system brings this idea into the realms of possibility, though as yet, of course, the number of projectors available is small and their cost relatively high.

The demonstration at Belle Vue involved the transportation of two large van-loads of equipment and a team of Swiss experts to Britain, and clearly involved a great deal of work for the organizers; the result certainly justified the effort. C. A. Taylor

INSECTS AND LENGTH OF DAY

Stanley D. Beck, of the University of Wisconsin, has studied the effect of temperature on the hibernation of the European corn borer, a species of moth that passes the winter not as an egg but as a mature larva (*Sci. American*, 202, February 1960). The corn borer is a long-day insect. Generation after generation can be reared in the laboratory if the cultures are maintained under any of three different conditions: 16 hr. of light a day, continuous darkness or continuous light. In the absence of suitable photoperiod—that is, in continuous light or darkness—temperature has only a slight effect on the incidence of hibernation. Similarly, if a batch of borer larvæ are exposed to short photoperiods of 10·5–13·5 hr. of light a day, they will go into diapause regardless of the temperature at which they are reared. At photoperiods of 8–10 hr. and 14–15·5 hr., however, temperature exerts a powerful influence on the incidence of hibernation: high temperatures tend to prevent it and low temperatures to increase it.

This sensitivity to temperature is important in the life-cycle of the borer. It explains why two generations of them are produced each season in southern corn-growing areas, while only one generation is produced in northern areas. If photoperiod were the only factor governing their reproduction the opposite would be expected, because on any given day in summer the period of daylight is shorter in the south than in the north. The higher temperatures of the earlier southern growing-season, however, more than compensate for the shorter days.

In contrast to the silkworm and the aphid, which are sensitive to photoperiod only in the early stages of development, the borer remains responsive for a longer time. Present evidence indicates that it becomes sensitive in the later stages of larval development or is uniformly sensitive throughout the entire period of growth.

Photoperiod affects members of the growing generation of corn borers but not their progeny. There is no carry-over to the next generation as in the silkworm and the aphid. In the corn borer the earliest detectable symptom of impending diapause is a failure of the reproductive organs to develop. At a stage when the sex cells are rapidly maturing in borers reared under long photoperiod, these cells have ceased to develop in larvæ reared under short photoperiods. The short-photoperiod borers still feed and grow rapidly, but the growth of their reproductive organs is arrested. Similar gonadal arrest has been observed in a number of other species of hibernating insects, including such diverse types as boll weevils and Colorado potato beetles.

In insects, as in higher animals, growth and gonadal development are controlled by hormones. The insect growth-hormone has been identified as ecdysone, which is produced by glands in the insect's thoracic tissue. Insects in diapause contain no detectable amounts of ecdysone, but if they are injected with it, they soon resume normal development. It appears that the change in photoperiod inhibits the production of ecdysone. How this inhibition is brought about is not known. Perhaps a diapause hormone is responsible, especially in forms like the aphid and silkworm. If this is so, the insects must be sensitive to the hormone only at certain times during their lives.

Another unresolved issue is the nature of the timing mechanism that enables an insect to distinguish between a 16-hr. photoperiod and one of, say, 12 or 13 hr. A light receptor is required, because the insect must discriminate between light and dark to respond to photoperiod at all. Apparently the eyes are not involved, and the shielding of different parts of the insect's body has not located any other organ or nerve ending that is specially sensitive to photoperiod. All parts of the body seem

equally sensitive. It now seems likely that the receptors are either numerous cells scattered over the body surface, or that they are the cells of the integument itself.

The quantity of light involved has little to do with the photoperiod reaction, so long as the intensity is greater than a very low threshold value—usually less than 1 ft.-candle. This suggests that no photochemical reaction is involved, for the rate of most photochemical processes increases with greater light intensity. It is possible that a small-scale photochemical reaction may act as a trigger; in that case the most important cause of the photoperiod response would not be the presence or absence of light, but the stimulus produced by the change from light to dark, and vice versa. Each 24-hr. day involves one change of light to dark, and one change from dark to light, but these changes can provide no more than a series of stimuli that start a biological switch. Because different day-lengths induce different responses, the switch presumably activates the biological clock. The clock measures the changing interval between stimuli and regulates the physiology of the insect accordingly.

The existence of such a clock can easily be demonstrated in the European corn borer. When a batch of borers are reared under photoperiods of 12 hr. of light and 12 hr. of darkness, all of them go into hibernation. The ratio of light to dark in this case is one to one. When an artificial 12-hr. day is created with a one to one ratio of six hours of light and six hours of darkness, none of the borers hibernates. A 'day' of at least eight hours of darkness is apparently required to induce hibernation.

PAPER CHROMATOGRAPHY: MULTIPLE SPOT FORMATION BY PURE SUBSTANCES

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PURE pharmacologically active amines give more than one spot on paper chromatograms in the presence of certain acids (unpublished observations). Because this phenomenon lacked rationale and has important implications in relation to metabolic studies, we have examined the paper chromatographic behaviour of some amines and amine salts in detail. Using acidic (and neutral) solvent systems, we have shown that double amine spot formation on paper chromatograms is dependent on three major factors: the relative R_F values of the individual acids and bases of the salt, the relative concentrations of the acids and bases and the relative dissociation constants of the added acid and that present in the solvent system.

The chromatographic behaviour of monoacidic amines in a water-saturated *n*-butanol–acetic acid solvent system is summarized as follows: a single amine spot, having an R_F value similar to that of the free base, is obtained when weak acid salts (for example, propionates, mandelates, citrates) of many amines (for example, adrenalin, codeine, amphetamine, propylamine) are chromatographed. With an organic acid salt, in which the organic acid has a low pKa (less than 1) and a high R_F value (for example, trichloracetic acid pKa 0·65, R_F 0·90), two amine spots are obtained unless the amine and acid have similar R_F values (for example, chlorpromazine trichloracetate, R_F of the base 0·92) or the amine is very insoluble in water (for example, analgesics based on the piperidine structure[1]). Of the two amine spots, one has an R_F value corresponding to that of the free base while the other is associated with the acid spot. The position of the latter amine spot is dependent upon the R_F value of the acid. Thus, the separation of the amine into two spots and their relative positions is dependent on the relative R_F values of the components. The results for various amine trichloracetates are represented diagrammatically in Fig. 1.

With salts showing double amine spot formation, chromatography of the salt in the presence of an excess of the acid results in a greater proportion of the base becoming located in the spot associated with the acid. A single amine spot is obtained if a sufficient quantity of acid is used, the amount of acid required being dependent on the difference between the R_F values of the individual components. However, amines of low R_F value (for example, noradrenalin and adrenalin, R_F 0·34 and 0·42) always formed two amine spots in the presence of trichloracetic acid even using a twenty molar excess of the latter. Increasing the acid concentration also results in the R_F value of the amine spot associated with the acid approaching that of the free acid, whereas the R_F value of the second amine spot (if still present) is analogous to that of the free base.

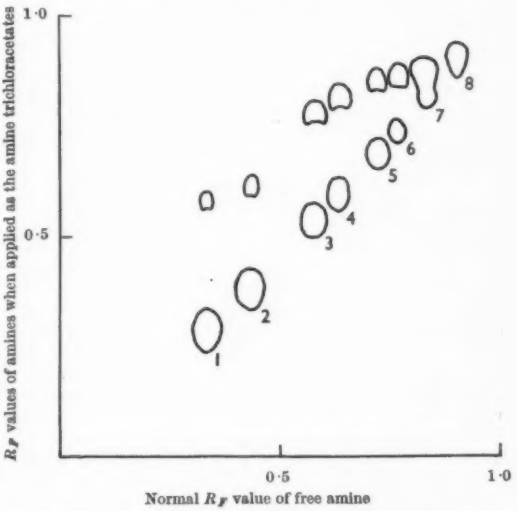

Fig. 1. Chromatograms of amine trichloracetates (about 20 μgm.) run in *n*-butanol–acetic acid–water (4 : 1 : 5) on Whatman No. 1 paper. Tailing is not shown. 1, noradrenalin; 2, adrenalin; 3, propylamine and codeine; 4, tyramine; 5, β-phenylethylamine; 6, ephedrine and amphetamine; 7, papaverine; 8, chlorpromazine

Tailing between the two base spots was most marked where the R_F values of the components were widely separated and with the salts of weaker acids. It became less obvious if smaller amounts of the amines were applied to the paper when discrete spots may be obtained, that is, the detection of tailing is a function of reagent sensitivity.

Salts of bases and organic acids in which the R_F value of the free base is greater than that of the free acid yield a single amine spot associated with the acid, unless the R_F values of the components are widely separated (for example, N-phenethyl 3-methyl 4-(*o*-tolyl)4-acetoxypiperidine trichloracetate R_F of the base is 1·0) when complete acid/base separation occurs. Examples are ephedrine maleate (R_F 0·77; ephedrine, R_F 0·76; maleic acid, R_F 0·71) and ephedrine picrate (R_F 0·88; picric acid, R_F 0·74). In contrast, propylamine maleate yields two amine spots (R_F 0·56 and 0·67 with tailing in between; propylamine, R_F 0·59).

Although double amine spot formation depends partly upon the relative dissociation constants of the acids present in the salts and in the solvent, the proximity of the R_F values of the acids and bases is the governing factor with the salts of weaker organic acids (*pKa* values of 1–3). If the difference between the R_F values of the acids and bases is small, two amine spots are obtained (for example, propylamine, isoprenaline and phenylethylamine dichloracetates, R_F values of the bases are 0·59, 0·60 and 0·73, respectively, and the R_F value of dichloracetic acid is 0·83. Complete separation of the base from the acid, with consequent single amine spot formation, occurs if the R_F values of the acid and base are widely separated (for example, noradrenalin and adrenalin dichloracetates).

The shapes and R_F values of amine spots are modified by inorganic acids such as sulphuric and hydrochloric acids (R_F 0·39 and 0·38, respectively). Elongated amine spots are produced by the sulphates and hydrochlorides of noradrenalin and adrenalin; the acid spots overlap those of the bases. Using excess of sulphuric acid but not hydrochloric acid both these substances formed spanner-shaped amine spots; the acid was located in the cup and also overlapping the base of the amine spot. Amines of intermediate R_F values (for example, propylamine) formed inverted crescent-shaped spots at the base of the main acid spot in the presence of an excess of a mineral acid. Complete acid/amine separation occurred with the sulphates and hydrochlorides of phenylethylamine and atropine (R_F 0·78) and normal amine spots were obtained. In contrast, phenylethylamine and ephedrine dissolved in an excess of trichloracetic acid produced crescent-shaped amine spots at the apices of the acid spots while, under the same conditions, codeine (R_F 0·57) and isoprenaline appeared as half-moon-shaped spots in the upper area of the acid spots.

Ghost spots[2] were observed with all phenolic amines in the presence of excess of any of the stronger acids.

In a neutral solvent system (water-saturated *n*-butanol) *all* except the more volatile acids profoundly affected the R_F values of the amines; acid/base separation of the salts occurred, but to a lesser extent than in the acidic solvent. If the R_F values of the base and acid were close (and the R_F value of the latter was greater than that of the former) one amine spot, associated with acid, was detected having an R_F value similar to that of the acid. With a greater difference between the R_F values of the individual components two amine spots were produced, whereas, with widely varying R_F values, complete acid/base separation occurred and a single amine spot was observed.

Using a basic solvent system (water-saturated *n*-butanol–ammonia) the R_F values of the amines, as expected, remained unchanged in the presence of any of the acids tested.

Multiple spot formation is not necessarily indicative of the presence of different substances. The present work re-emphasizes that reference substances and unidentified materials must be chromatographed under identical conditions, if valid deductions are to be drawn. This is particularly important with amines in biological extracts which may also contain certain organic acids, or to which trichloracetic acid has been added. Even when free bases are liberated with alkali and extracted and chromatographed, the presence of trace amounts of acid or salts carried over during extraction can cause multiple spot formation from single substances. Similar remarks also apply to the chromatographic examination of acids in basic solvents.

Thus the chromatographic investigation of the metabolism of basic drugs may readily yield more spots than there are metabolites if strongly acidic compounds are also products of metabolism. The addition of bases (for example, ephedrine) to urine, for example, may yield more than one base spot in neutral running solvents because of the presence of acids.

This and related work with other systems will be described in detail elsewhere.

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DISPROPORTIONATION AND DIMERIZATION OF THE BENZYLOXYL FREE RADICAL IN SOLUTION

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ALTHOUGH the mutually competitive processes of disproportionation (1) and dimerization (2) of alkoxyl radicals:

$$2RCH_2O\cdot \longrightarrow RCHO + RCH_2OH \quad (1)$$

$$2RCH_2O \longrightarrow RCH_2O{-}OCH_2R \quad (2)$$

have been much discussed from the thermochemical point of view[1,2] actual evidence in support of (1) and (2) is somewhat scanty.

Disproportionation for various alkoxyl radicals is regarded as a highly exothermic process. Gray[1] has assigned enthalpies in excess of 80 k. cal. mole^{-1}, and

activation energies are expected to be very small or zero. Reaction (1) has been postulated to explain certain features of the decomposition of diethyl peroxide in presence of ethyl nitrite and nitric oxide[3]. The presence of benzaldehyde in the decompositions of benzyl nitrite and dibenzyl hyponitrite in solution has been considered by Gray[1] as evidence for disproportionation. However, this must await a kinetic study in order to ascertain the importance of induced reaction which may also yield benzaldehyde by the following reactions:

$$R\cdot + PhCH_2O{-}N=N{-}OCH_2Ph \longrightarrow \begin{matrix} RH + PhCHO{-}N=N{-}OCH_2Ph \\ \text{or} \\ Ph{-}\dot{C}HO{-}N=O \end{matrix} \quad \text{or} \quad PhCH_2O{-}N=O \qquad (3)$$

$$\begin{matrix} PhCHO{-}N=N{-}OCH_2Ph \longrightarrow PhCHO + N_2 + PhCH_2O\cdot \\ PhCHO{-}N=O \longrightarrow PhCHO + NO \end{matrix} \qquad (4)$$

Wijnen[4] has considered evidence for disproportionation of methoxyl radicals in the photolysis of methyl $-d_3$ acetate at 30° and 90°.

At these comparatively low temperatures no evidence to support reaction (2) was available. Evidence for dimerization in solution is quoted for the 2-phenyl propan-2-oxyl radical by Gray[1] and Walling[5]:

$$2Ph{-}C(Me)_2 - O\cdot \longrightarrow (Ph{-}CMe_2{-}O)_2 \qquad (5)$$

The work actually referred to is that of Kharasch *et al.*[6], who themselves doubt whether the peroxide is formed by dimerization. This case should be favourable to dimerization as competition from disproportionation is absent. Dimerization should be exothermic to the extent of 35–40 k. cal. mole^{-1}. The kinetics of thermal decomposition of dibenzyl hyponitrite in solution has been studied by us. The decomposition is first-order for at least 75 per cent of complete reaction. Induced reaction is, however, evident as the rates are lowered by presence of inhibitors such as quinones, and vinyl monomers. At sufficiently high concentration of *p*-benzoquinone the rate becomes independent of quinone concentration and the decomposition is first-order throughout the range of accurate observation (more than 90 per cent decomposition). The activation energy for this first-order decomposition is $24{\cdot}9 \pm 0{\cdot}25$ k. cal. mole^{-1}. This low value suggests a simultaneous rupture of both O—N bonds resulting in the elimination of a nitrogen molecule and the formation of two benzyloxyl radicals in a 'cage'[7]. Dibenzyl hyponitrite is known to polymerize methyl methacrylate[8]. We also have evidence to support radical intermediates in the decomposition of dibenzyl hyponitrite in solution:

$$PhCH_2O{-}N=N{-}OCH_2Ph \longrightarrow 2PhCH_2O\cdot + N_2 \qquad (6)$$

The products isolated from solutions were benzaldehyde (as 2 : 4-dinitrophenylhydrazone) and benzyl alcohol (as 3 : 5-dinitrobenzoate) in high percentage yields. In *cyclo*hexane solutions the benzaldehyde is readily detectable by its maxima at 240, 276 and 285·5 mμ. At 285·5 mμ benzaldehyde absorbs strongly ($\varepsilon = 1{,}000$) whereas benzyl alcohol absorption is negligible ($\varepsilon = 2$) and this maximum was used for quantitative analyses for benzaldehyde. As the induced reaction is a second-order reaction it should be less important at high dilutions whereas the 'cage' reactions are independent of concentration. A study of benzaldehyde yield for various concentrations is shown in Table 1.

The yield drops from 65 per cent at 0·1 *M* to 50 per cent at 0·001 *M* and remains stationary thereafter to the extreme dilution studied of 0·0001 *M*. The high and constant yield of benzaldehyde at high dilutions strongly supports the formation of benzaldehyde solely by a 'cage' disproportionation. A pair of benzyloxyl radicals formed in a 'cage' by decomposition of a molecule of dibenzyl hyponitrite may undergo disproportionation, dimerization or separate by diffusion. Benzaldehyde may thus be formed through a reversible fission of dibenzyl peroxide if any is formed. The half-life of dibenzyl hyponitrite at 35° in *cyclo*hexane is 2·46 hr.

Dibenzyl peroxide does not appear to be known; it should have approximately the same half-life as comparable peroxides. In solution di-α-cumyl peroxide would have a half-life of $1{\cdot}26 \times 10^5$ hr. at 35° (by extrapolation)[9]. Thus at 35° it would appear that benzaldehyde should be formed directly from the disproportionation of benzyloxyl radicals generated by the parent hyponitrite.

A study of the rate of appearance of benzaldehyde in *cyclo*hexane solutions using the 285·5 mμ maximum was attempted. The optical density increased rapidly though the kinetics were not strictly first-order. This may be due to the fact that dibenzyl hyponitrite has a small but finite absorption at 285·5 mμ ($\varepsilon = 20$). Half the final yield of benzaldehyde is formed in 2·75 hr. This compares with 2·46 hr. for the half-life of dibenzyl hyponitrite in *cyclo*hexane at 35°. After several half-lives at 35° the yield of benzaldehyde remains constant (Table 2).

Thus the yield after 96 and 151 hr. at 35° is the same (49 per cent), indicating that dibenzyl peroxide if present does not appear to give benzaldehyde in this

Table 1. Percentage Yield of Benzaldehyde in the Decomposition of Dibenzyl Hyponitrite in *cyclo*Hexane at 40° C.

Concentration of solution (mole/l.)	Per cent C_6H_5CHO
0·1000	65·34
0·0500	65·58
0·0100	57·38
0·00748	55·80
0·00500	53·15
0·00453	53·47
0·00399	53·50
0·00351	52·91
0·00301	53·95
0·00198	53·02
0·00149	51·29
0·00100	50·55
0·000500	50·29
0·000103	50·29

Table 2. Benzaldehyde Analyses (in Duplicate) for 0·00076 *M* Solutions of Dibenzyl Hyponitrite in *cyclo*Hexane after Decomposition

	Optical density			Per cent C_6H_5CHO
	Sample *A*	Sample *B*	Average	
96 hr. at 35°	0·378	0·374	0·376	49·01
96 hr. at 35° + 65 hr. at 35°	0·379	0·373	0·376	49·01
96 hr. at 35° + 65 hr. at 100°	0·419	0·420	0·420	54·74

period of time. However, subjecting solutions which had undergone decomposition for 96 hr. at 35° to a further 65 hr. at 100° C. (where the calculated half-life of dibenzyl peroxide is 55 hr.) increased the yield of benzaldehyde by 5 per cent. The reverse of reaction (2) followed by disproportionation (1) is indicated.

It appears that benzyloxyl radicals undergo disproportionation and dimerization in a 'cage', and their relative rates may be thus studied. Experiments are being undertaken aimed at isolation of dibenzyl peroxide as well as detection, product and kinetic studies by gas chromatography and infra-red spectroscopy.

One of us (S. K. H.) is grateful to the Education Department, Hong Kong, for permission to participate in this work.

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SYNTHETIC POLYELECTROLYTES AS TUMOUR INHIBITORS

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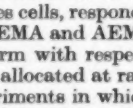
ANIONIC polysulphonates and polyphosphates of either natural or synthetic origin have been shown to be potent inhibitors of transplanted tumours in mice[1-4]. This inhibition of tumour growth may conceivably be a function of the density and distribution of ionic charges within the polyelectrolyte molecule. To test this hypothesis, the tumour-inhibiting properties of synthetic polymers of varied charge-densities were studied.

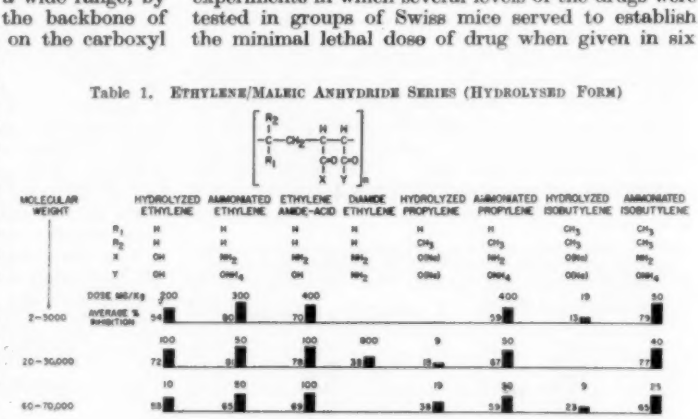
Two classes of synthetic polymers were chosen: polycarboxylates derived from ethylene/maleic anhydride copolymers and those derived from polyacrylic acid. (For convenience, in the ethylene/maleic anhydride copolymer (EMA) series, hydrolysed (free carboxyl) form is abbreviated HEMA and the ammoniated (amide-acid form) is noted as AEMA.) The charge-density and solution configuration of these compounds were varied over a wide range, by proper choice of substituents on the backbone of the molecule and by substitutions on the carboxyl groups. In the ethylene/maleic anhydride copolymers, functional groups are attached to immediately adjacent carbon atoms of the ethylene backbone, whereas in the polyacrylic copolymers functional groups are attached to backbone carbon atoms separated by a methylene unit. Because of this difference, the two classes of polymers differ in ion strengths as well as charge distribution. For each class of polymer two different series were studied: the free dicarboxylic acids and the carboxamide–carboxyl types, each in several molecular-weight ranges. The effect of these compounds on the growth of transplanted tumours in mice is reported in this article.

In most experiments, sarcoma 180 was the tumour used. Additional experiments were conducted with Krebs 2 carcinoma, leukæmia *L*-1210 and carcinoma 755, maintained and tested in appropriate host strains[2].

Sarcoma 180, Krebs 2 carcinoma or leukæmia *L*-1210 ascites tumour cells, grown for 5–7 days in the donor mouse, were injected subcutaneously into the flank of test animals as a 0·2-ml. suspension of approximately 5,000,000 cells. Non-necrotic solid sarcoma 180 or carcinoma 755 tumour, 6–14 days old when transplanted, was placed by trocar into the flank of the appropriate host as a fragment approximately 2 mm. in diameter. Solid tumour, derived from either solid tumour or subcutaneously placed ascites cells, responded in essentially the same manner to HEMA and AEMA. In all experiments, mice were uniform with respect to sex, age and weight. They were allocated at random 3–5 to a cage. Preliminary experiments in which several levels of the drugs were tested in groups of Swiss mice served to establish the minimal lethal dose of drug when given in six

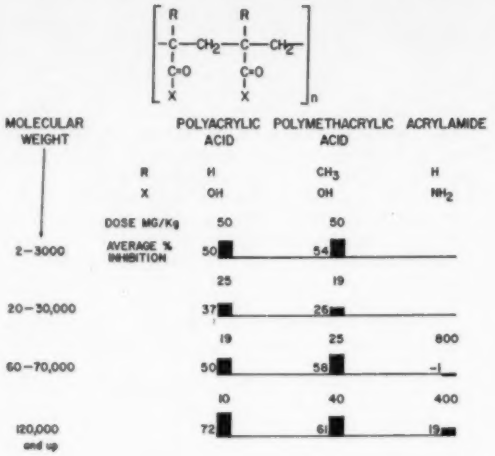
Table 1. Ethylene/Maleic Anhydride Series (Hydrolysed Form)





INHIBITION OF SUBCUTANEOUS SARCOMA 180

Table 2. POLYACRYLIC AND POLYMETHACRYLIC SERIES



daily doses. The final experimental groups consisted of a minimum of 10 mice.

Solutions of the compounds to be tested were prepared in isotonic saline and stored in the cold. If necessary, *p*H was adjusted to approximately 7 by titration with dilute sodium hydroxide.

Treatment consisted of a single daily intraperitoneal injection of 0·5 ml. of drug solution, beginning 24 hr. after tumour inoculation. Injections were given for 6 days and animals killed the eighth day after tumour inoculation. The same method was followed with carcinoma 755 except that the tumour was permitted to grow for 7 days prior to start of treatment. Each tumour was removed by dissection, weighed and compared with the tumour-weight of saline-treated control animals.

Experiments in which more than one-third of the treated animals died were not considered valid. Using this criterion of validity, consistent inhibition of sarcoma 180 from 50 to 83 per cent was noted in 41 consecutive experiments with AEMA of different molecular weights. In 19 of these experiments, no animals died. Thus, tumour inhibition is not necessarily a manifestation of non-specific toxicity. For the remaining 33 compounds, single experiments are presented. In 23 of these experiments, inhibition of sarcoma 180 tumour growth of 50 per cent or more occurred. Other experiments at different dose-levels with each of the 33 compounds confirm the general order of tumour inhibitory activity, usually with excessive toxicity when the dose was raised.

Body-weights were recorded at the beginning and end of each experiment. For all experiments, total gain in body-weight of the control mice averaged 3·9 gm. and that for the treated mice 1·9 gm. Loss of weight was found in only 8 out of 64 experiments where the inhibition of sarcoma 180 was 50 per cent or greater, and in only 3 of these experiments did the losses exceed 10 per cent of the pre-treatment weight.

Survival-time in mice bearing ascites tumour was determined after intraperitoneal inoculation of a uniform suspension containing approximately 2,000,000 ascites tumour cells. 24 hr. after tumour inoculation, AEMA or HEMA was given for six consecutive days either intraperitoneally or subcutaneously. Survival-time of these mice was contrasted with control mice treated in similar fashion with saline.

AEMA (mol. wt., 2–3,000 and 20–30,000) given intraperitoneally to mice with Ehrlich ascites tumour clone 2 or leukæmia *L*-1210 ascites and AEMA and HEMA (mol. wt., 20–30,000) given subcutaneously to mice with leukæmia *L*-1210 ascites and Krebs 2 carcinoma ascites did not increase survival-times.

Results in Tables 1 and 2 are presented as the average per cent inhibition of sarcoma 180 determined at dose-levels which range from just less than the minimal lethal dose to a dose which kills no more than one-third of the animals.

Table 1 includes the following maleic anhydride copolymers: the dicarboxylic acid–HEMA, the ammonium salt of AEMA, the amide-acid form of AEMA, the EMA diamide and hydrolysed and amidated propylene and *iso*butylene maleic anhydride. Molecular weights ranged in five groups from approximately 2,000 to 120,000 as estimated from measurements of solution viscosity. Where possible, each compound was tested for inhibitory activity in each of the five weight ranges.

It appears that at least one ionizable carboxyl group is required for the polymers to manifest significant tumour inhibition. The diamide of EMA showed a much lower order of both inhibitory activity and toxicity than HEMA or AEMA. Lethality of the dicarboxylic acid HEMA increased as the molecular weight increased up to 80–100,000. The dicarboxylic acid propylene and *iso*butylene copolymers tested were more toxic, and less active as tumour inhibitors against sarcoma 180 than the corresponding half-amide acid compounds.

Analogous results for polyacrylic acid, polymethacrylic acid, and polyacrylamide are presented in Table 2. In this series, also, the completely amidated product was not significantly effective as a tumour inhibitor and was much less toxic than carboxyl-containing compounds of similar molecular weight. In general, tumour inhibition by polymers of this series was similar to that of corresponding polymers of the series shown in Table 1. Based on the results obtained, the difference between the two series in position of carboxyl groups on the ethylene backbone does not alter greatly the tumour-inhibiting property of the compound.

Fig. 1 presents a comparison of the inhibiting action of low molecular weight (2–3,000) HEMA and AEMA against several tumours. At equivalent doses, monocarboxylic AEMA copolymer was effective against sarcoma 180, leukæmia *L*-1210, Krebs 2 carcinoma and carcinoma 755. Dicarboxylic HEMA

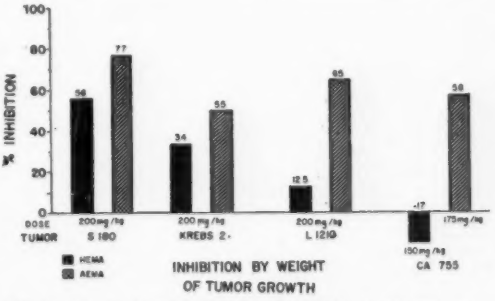


Fig. 1. Comparison of HEMA and AEMA 2–3,000 mol. wt. against a variety of transplanted tumours

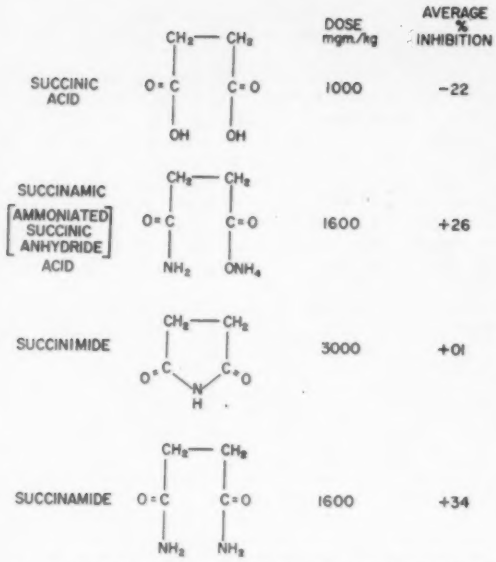


Fig. 2. Inhibition of sarcoma 180 by monomeric fragments of the ethylene/maleic anhydride series

copolymer inhibited only sarcoma 180. This same pattern of inhibition was observed for AEMA and HEMA in molecular weight ranges of 20–30,000 and 60–70,000. AEMA thus showed a broader spectrum of tumour inhibition than HEMA and is the more potent inhibitor of sarcoma 180 at equitoxic doses. (A comparative study with analogous derivatives in the acrylic acid series is being completed.)

The corresponding monomeric fragments of HEMA and AEMA such as succinic acid, succinamic acid, succinimide and succinamide, administered at levels of 1,000–3,000 mgm./kgm., did not show significant inhibitory activity against sarcoma 180 (Fig. 2). These results indicate the necessity for the polymeric structure in producing the observed tumour inhibition.

Tumour inhibition for the two polymer series reported (completely carboxylated and carboxyl–carboxamide types) was strongly dependent on carboxyl substitution, which varied molecular configuration in solution. Within each series, charge-density is one factor which may govern tumour-inhibiting effectiveness and toxicity.

Optimum activity was obtained in the series where carboxamide and ionizable carboxyl groups were interdispersed on the polymer backbone. When all carboxyl groups are converted to carboxamides, significant tumour inhibition was lost. In addition, proper carboxamide–carboxyl balance broadens the scope of activity against a variety of transplanted tumours. Within this series, amide-acids of either ethylene, propylene or *iso*butylene maleic anhydride copolymers exhibited equivalent tumour inhibitory activity at equitoxic dose-levels.

For the completely carboxylated polymers those of weaker degree of ionization (hydrolysed propylene and *iso*butylene maleic anhydride copolymers) were, in general, less active than those of higher charge-density (HEMA, polyacrylic and polymethacrylic acids).

The results of our studies support the hypothesis that tumour inhibition by polyelectrolytes may be a function of charge-density and distribution on the polyelectrolyte molecule. The inactivity of the monomeric fragments tested indicates the necessity for the polymeric structure.

Synthetic polyelectrolytes not only inhibit transplanted tumours but also certain vital enzymes such as ribonuclease and deoxyribonuclease[3,5,6]. The exact mechanism for these effects is not known. The variation which is possible in the physico-chemical characteristics of synthetic polyelectrolytes permits us empirically to define certain properties contributing to tumour inhibition. Predictable reactions between synthetic polyelectrolytes and selected biological systems may eventually be possible by consideration of physico-chemical characteristics of the synthetic and the naturally occurring polyelectrolytes.

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EFFECT OF OXYGEN AND NITRIC OXIDE ON THE RADIOSENSITIVITY OF HUMAN CELLS IN TISSUE CULTURE

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THE sensitivity of cells to radiation can be measured by several different criteria. From the point of view of radiotherapy of cancer, the relevant criterion is a loss of reproductive integrity, that is, the loss of the ability of the cell or its descendants to go on multiplying indefinitely. The work of Puck and his collaborators has shown that it is feasible to measure this form of radiation damage to human cells in tissue culture by observing the fraction of cells that survived, in that they were able to go on dividing and growing to form a visible colony. The radiation procedure used by Puck and Marcus[1] had the disadvantage of an uncertainty in dose, due to the close proximity of the cells to a glass surface[2]. This article reports the results obtained by an alteration of procedure that eliminates the glass surface, and at the same time permits an accurate control of the oxygen tension in the surrounding medium.

The cells used in this work were from human liver, obtained in tissue culture by Westwood[3] and kindly

supplied to me by Dr. F. Sheffield. They were grown in closed bottles containing Earle's salt solution, lactalbumin hydrolysate, tryptic meat broth, serum, glucose, antibiotics and bicarbonate—the mixture having been pre-gassed with carbon dioxide. Before irradiation they were removed from the glass surface by incubating with 0·5 m*M* ethylene-diamine-tetraacetic-acid disodium salt for 15 min., then pipetted rapidly to break up clumps. One fraction of the suspension was used for microscopical examination for total cell-count and for a check against clumping. Other fractions were added immediately to phosphate-buffered media in which the bicarbonate had been omitted and replaced by 8 per cent by volume of buffer at *p*H 7·4, made by adding 4 volumes of 0·154 *M* disodium hydrogen phosphate to 1 volume of 0·154 *M* sodium dihydrogen phosphate.

The cells were irradiated in suspension in phosphate buffer in a 'Perspex' ('Lucite') vessel. The solution was bubbled with the gas under test as fast as foaming would permit, for at least 5 min. before, and during irradiation. The radiation used was from a Siemens 'Metalix' X-ray tube operated at 200-kV. constant potential, 8 m.amp., and filtered by 0·5 copper + 0·5 mm. aluminium. The cells were irradiated at room temperature, at a dose-rate of about 600 r./min. 0·1 ml. samples of cell suspension were withdrawn from the vessel before and after irradiation, diluted as necessary, and aliquots placed in closed bottles containing bicarbonate medium, carbon dioxide and an actively metabolizing X-rayed 'feeder layer'. Colonies were counted after 10 days incubation at 37° by scanning the bottle under the low-power objective of a microscope. A ruled grid was placed over the surface of the bottle during counting to ensure that the surface was scanned once, and only once.

The effect of X-rays on the viability of cells suspended in media gassed with oxygen, nitrogen, and 0·275 per cent oxygen in nitrogen, is shown in Fig. 1. The errors shown represent only the statistical error of counting and therefore under-estimate the total error of each point. The lines are the regression lines determined by 'least-square' analysis after giving each point a weight determined by the number of cells counted. The air value at 18·3 per cent survival was the first result to be obtained. Since a slightly different geometry was used during this run, the result has been omitted from the statistical calculations. The point itself has been included in the figure for completeness. The points for air and for 100 per cent oxygen were not significantly different, and both sets were put together in the regression analysis. The slope of the air–oxygen line corresponds to a 37 per cent survival dose of 118·8 ± 3·9 rads, and that of the nitrogen line to a dose of 263·1 ± 16·6 rads. Thus the oxygen–nitrogen ratio m is 2·2.

Alper and Howard-Flanders[4] found that the sensitivity of bacteria to X-rays at different oxygen concentrations could be fitted by the equation:

$$S(O_2) + SK = S_n m(O_2) + S_n K$$

where S_n is the radiosensitivity in the absence of oxygen ($= 1/D_{37}$ dose for nitrogen). S is the radiosensitivity at oxygen tensions (O_2) and m and K are constants. The same equation was found to apply for Ehrlich ascites cells by Deschner and Gray[5]. If these human cells are assumed to obey this equation, then $K = 8·5$ μmoles/l., the same order of magnitude as was found for bacteria (7·8) and for mouse tumour cells (5 ± 2). A value of 4·2 has also been reported[6] for bean roots poisoned with cupferron. The plating efficiency varied between 40 and 70 per cent from one experiment to another, but was remarkably constant over all the controls in any one experiment. The experimental procedure alone, including filling the irradiation vessel, gassing with nitrogen for the longest periods used, and all other operations except switching on the X-ray set, gave the same survival as controls which were not subjected to these procedures.

During the cell count a record was also kept of the number of cells in the counting field that appeared to be touching one another. This was usually about 10 per cent of all cells counted, and is the sum of cells actually stuck together and cells which happen to be lying very close to one another. In the worst case, in which this proportion of cells remained in contact throughout the experiment, then the error from this cause would be 5 per cent, or about equal to the counting error. In fact the cells, when plated in bottles, are a thousand times more dilute than in the cell-counting chamber. Thus, the error due to cell clumping would be expected to be very much less than the statistical errors indicated in Fig. 1. The irradiated cell suspension was relatively dilute, between 10^4 and 10^5 metabolizing cells per ml. The total oxygen consumption of the cells would not make a measurable difference to the oxygen tension of the medium, during irradiation in oxygen or air. On the other hand, no help from the cells can be expected during equilibration with nitrogen for the anoxic radiations. Any error here would tend to make the value of m too small, and K too large. In order to assess the magnitude of these errors, aliquots of the equilibrated anaerobic solution were transferred to a Hersch cell[7] and the residual oxygen measured.

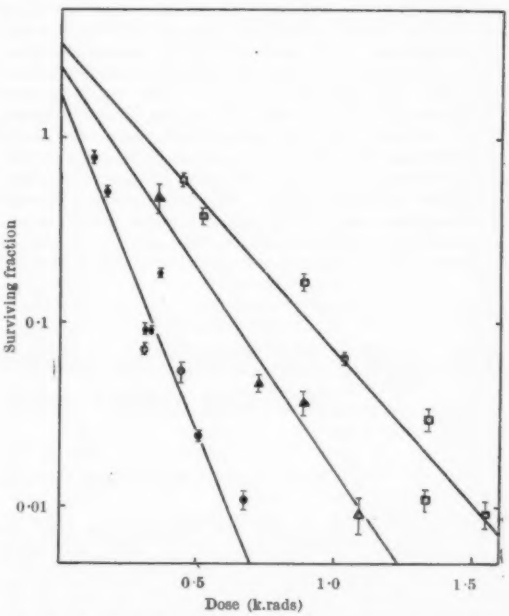

Fig. 1. Survival of human liver cells after 200-kV. X-rays during equilibration with oxygen, ○, air ●, nitrogen □ and 0·275 per cent oxygen in nitrogen △. The lines are the regression lines determined by 'least-square' analysis

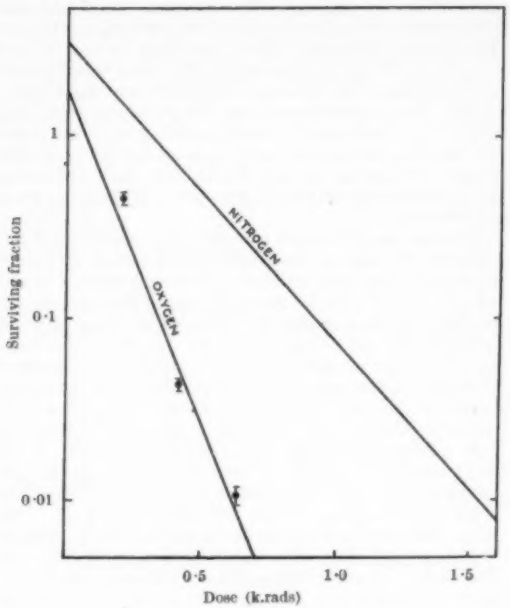

Fig. 2. Survival of human liver cells after 200-kV. X-rays during equilibration with 10 per cent nitric oxide in nitrogen. The oxygen and nitrogen lines are included for reference

These results indicated that the oxygen remaining in solution corresponded to equilibration with about 100 parts/million oxygen in the gas phase. Accurate measurement was made difficult by the foaming nature of the liquid; but if an upper limit of 275 parts/million is assumed then the corresponding value of m would be 2·4 and K would be 7·7 μmole/l. The feeder layer was made by giving bottles, containing a thick layer of growing cells, an exposure dose of 1,320 r. of 200-kV. X-rays. The medium was then poured off, the cells detached from the glass with ethylene-diamine-tetraacetic acid disodium salt, diluted in fresh bicarbonate medium and dispensed in clean bottles. The final concentration was about 10^5 cells per bottle. By extrapolating the air inactivation curve of Fig. 1, and applying the correction used by Morkovin and Feldman[3] for increased radiation-dose due to the proximity of the glass surface, then one colony in 10^2 bottles would be expected. This would introduce a negligible error.

The effect of adding 10 per cent nitric oxide during strict nitrogen anoxia is shown in Fig. 2. The lines are the oxygen and nitrogen lines from Fig. 1 and are included for reference. The conclusion from this experiment is that nitric oxide appears to act in a way similar to oxygen in sensitizing these human cells to X-rays, a phenomenon already found for bacteria by Howard-Flanders[6], for bean roots by Kihlman[9], and for Ehrlich ascites cells by Gray, Green and Hawes[10]. In dry systems nitric oxide acts in the opposite sense, in that it protects anaerobic spores of *Bacillus megaterium*[11] and dry seeds of *Agrostis stolonifera*[12] against damage by X-rays. The overall result is that with respect to the influence of oxygen and nitric oxide on radiosensitivity, human cells in tissue culture behave in a way similar to most other types of cell which have been studied. It is not suggested that cells in tissue culture are identical with those *in vivo*, or that results obtained by inactivating cells in culture can be applied indiscriminately to cells *in vivo*. The process of obtaining cells in tissue culture is by nature one of specifically selecting those cells best able to grow under those conditions supplied.

Nevertheless, the results reported here bear a striking resemblance to the results of irradiating leukæmia cells *in vivo* in live and dead mice reported by Hewitt and Wilson[13] and interpreted by them as the aerobic and anaerobic sensitivities.

I am indebted to Dr. Alma Howard for making the cell counts; to Dr. J. W. Boag, and Dr. R. E. Steele for help in the dosimetry; to Dr. L. H. Gray for suggesting the work and for encouragement and help, and to Mr. J. Steadman and Miss J. Liddon for technical assistance.

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INFLUENCE OF CERVICAL SYMPATHECTOMY AND PITUITARY STALK SECTION UPON MILK SECRETION IN GOATS

By G. B. TVERSKOY

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THE hormones of the adenohypophysis play an important part in control of milk secretion. During some days after the operation, hypophysectomy causes a cessation of lactation in spite of an injection of oxytocin—a neurohypophysis hormone, which stimulates milk ejection[1,2]. The control of the hormone-forming function of the adenohypophysis stimulating milk secretion is not yet quite clear. Very little is known about the role that the sympathetic innervation of the adenohypophysis and the nervous and vascular tracts of the pituitary stalk are taking in the control of the hypophysis function stimulating milk secretion. It was therefore considered desirable to examine the influence of cervical sympathectomy and pituitary stalk section upon milk secretion in goats[3–5].

Influence of Cervical Sympathectomy on Milk Secretion

In order to analyse the part the sympathetic innervation of the adenohypophysis is playing in control of milk secretion, the influence of a bilateral cervical sympathectomy upon the secretory process in the mammary gland was studied in an experiment with eight goats. Cervical sympathectomy was performed in two stages at an interval of one month. At each stage the upper and the middle cervical sympathetic ganglia were extirpated from one side, and a piece 2–3 cm. long was cut from the vertebral nerve at the point of its leaving the stellate ganglion. In two goats the stellate ganglia were extirpated from both sides instead of an extirpation of vertebral nerves. The common carotid artery and the jugular vein in the cranial part of the cervix were stripped of their adventitia along 3–3·5 cm. and the vascular walls were carefully treated with 5 per cent aqueous and alcoholic carbolic acid solutions. Two goats underwent a 2-stage control operation of cervical sympathectomy. Cervical sympathetic ganglia of these animals were not extirpated but only exposed.

A unilateral cervical sympathectomy led to a moderate and short decrease in milk secretion. For 10 days after the first operation stage the average amount of milk given by sympathectomized animals was 76·3 per cent of the initial level, while with control-operated goats it was 92·2 per cent. The initial level of milk secretion was achieved after the first operation stage both in control-operated goats and in most of the sympathectomized ones 20–30 days after the operation.

The extirpation of the cervical sympathetic chain from the other side led to a much more profound and prolonged inhibition of milk secretion. During the first 10 days after the second operation stage the average amount of milk given by sympathectomized goats averaged 59·7 per cent of the initial level, while with control-operated animals it was 90·5 per cent. Further, a gradual increase of milk secretion was recorded; but the milk secretion in sympathectomized goats increased extremely slowly. After the second operation stage, the average daily amount of milk given by sympathectomized goats in stall conditions (the animals kept on the same ration as that before the operation) was 80·8 per cent of the initial level, while with the control-operated animals it was 103·4 per cent. Transferring the goats to pasture resulted in an increase of milk secretion in all animals. However, in pasture conditions also the amount of milk secretion in sympathectomized goats (104·6 per cent of the initial level) was lower than in control-operated ones (136·0 per cent).

The above results show that bilateral cervical sympathectomy results in a significant and prolonged inhibition of milk secretion.

Together with a desympathization of the adenohypophysis the cervical sympathectomy causes a desympathization of the neurohypophysis, the thyroid and parathyroid glands[6–8], the hormones of which play an important part in the control of the motor and secretory function of the mammary gland. Special experiments on the measurement of the amount of the residual milk before and after the sympathectomy showed that a desympathization of the neurohypophysis does not cause such disturbances of the milk-ejection reflex, which could lead to an incomplete evacuation of the alveolar part of the gland and, consequently, to an inhibition of milk secretion. If before the operation the amount of the residual milk averaged in six goats 37·8 ml., after the sympathectomy it was 35·0 ml. Neither was the decrease in milk secretion after the cervical sympathectomy a result of a desympathization of the thyroid gland and the internal parathyroid glands. Special experiments showed that a simultaneous denervation of both lobes of the thyroid and of internal parathyroid glands does not result in a decrease in milk secretion. The average daily amount of milk during the first three 10-day periods after this operation—when the sympathectomized goats showed the lowest milk secretion—was, in goat No. 59, 98·6, 100·5 and 100·2 per cent of the initial level and, in goat No. 108, 100·1, 112·5 and 110·6 per cent.

Many authors have demonstrated by their investigations[9–15] that the cervical part of the sympathetic nervous system plays an important part in the control of the hormone-forming function of the adenohypophysis. It is quite possible that in our experiments the decrease in milk secretion after the cervical sympathectomy is due, first of all, to a disturbance of the production of glandular lobe hormones. The decrease of the hormone-forming function of the adenohypophysis could have taken place as a result of a desympathization of the glandular lobe itself as well as of a desympathization of various parts of the brain and, first of all, of the hypothalamus, which plays an important part in the control of the function of the adenohypophysis.

Influence of Pituitary Stalk Section on Milk Secretion

Various authors have investigated the influence of pituitary stalk section on the lactation process in rats and rabbits. These experiments proved that pituitary stalk section in lactating animals brought about starvation of their litters[16–18]. Evidently, it was a result of a disturbance of the milk-ejection reflex[19–21], as the mammary glands of the experimental animals contained during the first post-operative days a sufficient amount of milk[19,22]. If oxytocin was injected into female rabbits after a pituitary stalk section[23], their milk secretion remained at its initial level, although the growth of their litters was slower, evidently due to a change in quality of the milk. As oxytocin causes not only an evacuation of the alveolar part of the mammary gland but probably also leads to an increased production of prolactin[24], these experiments do not allow the conclusion that pituitary stalk section does not cause any decrease in milk secretion. Thus, the results obtained so far do not solve the problem of the influence of pituitary stalk section on the milk secretion process. Owing to this we investigated the influence of pituitary stalk section on the amount of milk secretion.

The operation of section of the pituitary stalk was performed in six goats during their first lactation. Section was carried out by the temporal approach[25]. Four goats underwent a control operation of stalk section. The pituitary stalk of these animals was exposed, but not cut. Three months after the operation all animals were killed and a serial histological analysis of the hypothalamo-hypophyseal region was performed (I. G. Urazov). The results of histological studies showed that three goats (Nos. 112, 313 and 143) underwent complete section of the pituitary stalk. Goat No. 143 showed, contrary to goats Nos. 112 and 313, a thick scar at the site of the

section, and its median eminence of the tuber cinereum was very seriously damaged. In three other goats pituitary stalk section was incomplete. Goat No. 146 underwent a complete section of the infundibular stem, whereas the glandular part of the stalk remained, to a great extent, intact. The infundibular stem in goats Nos. 322 and 319 remained intact, while the glandular part of the stalk was partially cut. All goats with a complete stalk section and goat No. 146 showed an atrophy of the neural lobe of the neurohypophysis. In goats Nos. 319 and 322 the neural lobe remained intact. The hypophysis of goat No. 143 was significantly decreased in size. All goats showed areas of atrophy in the anterior part of the glandular lobe.

In all goats with a complete section of the pituitary stalk and goat No. 146 the milk-ejection reflex disappeared after the operation, but it reappeared in 7–11 days. However, during some weeks after the operation the milk-ejection reflex was somewhat defective: a prolonged latent period of the reflex and some increase of the amount of residual milk were noted. Goats Nos. 319 and 322 (an intact infundibular stem, a partial section of the glandular part of the stalk) showed a full milk ejection reflex immediately after the operation.

We can now discuss the results of the investigations of the influence of a pituitary stalk section on milk secretion. During the first month after the operation (stall conditions, the same ration as that before the operation) the average daily amount of milk given by goats Nos. 112 and 313 (complete stalk section) was on an average 28·9 per cent of the initial level; goat No. 143 (complete pituitary stalk section with the formation of a thick scar, severe damage of the median eminence) gave 12·6 per cent, three goats with incomplete stalk section 40·5 per cent, and the control-operated animals 55·7 per cent of the initial level. All animals showed an increase of milk secretion on being transferred to pasture conditions. However, in pasture conditions also milk secretion in goats with stalk section was significantly lower than that in the control-operated animals. The average daily amount of milk during the whole post-operative period of investigation (80 days in stall and pasture conditions) was 48·2 per cent of the initial level in goats Nos. 112 and 313 (a complete stalk section), 21·6 per cent in goat No. 143 (complete stalk section with formation of a thick scar, severe damage of the median eminence), 67·1 per cent in three goats with an incomplete stalk section and 77·6 per cent of initial level in control-operated animals. If we assume that during 80 post-operative days the amount of milk secretion in control-operated goats expressed as a percentage of the initial level is 100, the amount of milk secretion in goats Nos. 112 and 313 will average 62·1 per cent in comparison with the milk secretion-level in control-operated goats, in goat No. 143 it will be 27·8 per cent and in three goats with incomplete stalk section, 86·5 per cent. Thus, a complete stalk section leads to significantly decreased milk secretion. Goat No. 143, which had a thick scar formed at the site of the section, and of which the median eminence of the tuber cinereum was severely damaged, showed a very marked decrease of milk secretion. An incomplete stalk section leads to a relatively small decrease of milk secretion.

These results prove that nervous and vascular tracts of the pituitary stalk play an important part in the control of the hormone-forming function of the adenohypophysis stimulating milk secretion.

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STRUCTURES CORRESPONDING TO TERMINAL BARS AND TERMINAL WEB IN MANY TYPES OF CELLS

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SURPRISINGLY little is known of the factors influencing the shape of cells. In the intestinal epithelium, two structures were found to have an important role in this respect: the terminal bars, which hold neighbouring cells in close contact, and the terminal web, a layer of fibrils extending across the cell apex between the terminal bars[1]. Interestingly enough, both these structures have the same staining and histochemical properties[1]; in particular, both are stained by successive treatment with tannic acid, phosphomolybdic acid, and amido black (referred to hereafter as the *TPA* technique). The question then arose as to whether cells other than those of the intestinal epithelium contained material with similar staining properties and perhaps with a similar role.

A variety of tissues and organs from male and female adult rats, as well as a few tissues from other animals, were fixed in Carnoy, dehydrated in alcohol and

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methyl benzoate, sectioned at 2μ and stained by the *TPA* technique[1,2]. A search was then made for structures staining like terminal bars and terminal web.

A terminal web which appeared in *TPA* preparations as a single layer extending between the terminal bars was seen not only in the chief cells of the intestinal epithelium (Fig. 1*A*) but also in cells of many other simple epithelia (Table 1, unilayered terminal webs). In all these cells, the terminal web appeared as a thin band located immediately below the apical surface. Using the polarization microscope, Schmidt[3] described a layer just below the striated border of intestinal cells, which exhibited a type of birefringence indicative of the presence of fibrous molecules parallel to the apical surface. Indeed, when sections were cut parallel to the free surface of the intestinal cells and stained with *TPA*, distinct fibrils were often seen within the terminal web.

A systematic survey of the literature revealed that, during the second half of the last century, the terminal web was described by a few authors[4–9], but no name was given to it. This structure was then forgotten, for, to Heidenhain, each unit of the striated border was equivalent to a cilium related to a centriolar granule, and a row of such granules crowded together would appear as a band[10]. This misinterpretation was widely accepted.

Some fifty years later, Sauer[11], who seemed to have been unaware of previous work on the subject, described "a structure which has the appearance of a stained membrane stretched across the end of the cell between the terminal bars" in the ependymal epithelium of amphibian embryos. He stated that this structure is composed of a network of fibrils of such dimension that they are usually not separately visible, and that it may exist in other columnar cells; he proposed the name 'terminal web'. Other authors who observed this structure did not distinguish it from terminal bars (as may be seen, for example, in Figs. 292 and 383 in ref. 12).

The terminal web of the intestinal epithelium was seen in electron microscope pictures of Palade and Porter (quoted in ref. 1) as a filamentous layer extending between the terminal bars and continuous with similar material present in the microvilli of the striated border. Similarly, Palay and Karlin[13] described this terminal web in detail as a dense feltwork of filaments stretched across the cell and from which other filaments extended into the units of the striated border and also into the cytoplasm.

Using the *TPA* technique, a careful examination of the terminal bars was carried out in 2-μ thick sections of the epithelial cells of the intestine cut parallel to the surface. Under these conditions the terminal bars did not appear to be the uniform rods classically described. Instead, they proved to be composed of juxtaposed granules, giving the appearance of a string of beads. Furthermore, the fibrils of the terminal web were seen to end in these granules. In view of the similarity of the granules with the desmosomes of the Malpighian cells in the epidermis, it was decided to refer to the terminal bar granules by the term 'desmosomes'[14]. Hence, the terminal bar consisted essentially of a series of desmosomes wherein web filaments were confluent.

In the cells of the proximal convoluted tubules of the kidney and the stereociliated cells of the epididymis, cross-sections of the unilayered terminal web appeared finely granulated. In the ciliated cells of the trachea, it was often possible to see a double row of granules connected to the cilia and held within a terminal web ending in desmosomes. Cilia and granules stained with *TPA* and could, therefore, be related to the web.

In the ameloblast, two unilayered webs were present, one at the apex and one at the base of the cell.

In other cell types, the terminal web may be multilayered, each layer being connected with a set of desmosomes. This was the case in the striated ducts of salivary glands (Fig. 1*B*, Table 1).

The terminal bar-terminal web complex is organized quite differently in some cells where fibres attached to the terminal bars extended from the apex of the cell towards the base in a pillar-like fashion. This was observed, for example, in the uterine epithelium. An extreme example of this arrangement was seen in the pillar and outer phalangeal cells of the organ of Corti, where the bundles of tonofibrils forming the pillars proved to stain with *TPA* (Fig. 1*C*).

Another arrangement was exemplified by the goblet cells of the intestinal epithelium or the surface mucous cells of the stomach in which the *TPA*-stained structure was lacy and basket-like, enclosing the mucous droplet (Fig. 1*D*). In addition, these cells had a pillar-like bundle of fibrils going from the terminal bars to the cell base (Fig. 1*D*).

A special type of terminal web was present in some exocrine glands, for example, in the acinar cells of the pancreas (Fig. 1*E*). The apex of the cell had a narrow rectangular surface, supported by a terminal web limited by terminal bars (composed of desmosomes). From these, fibrils extended into the cytoplasm in various directions to form an intricate network which outlined spaces enclosing the zymogenic granules. Such a complex web was not always restricted to the cell apex, but also extended laterally to the regions facing the intercellular canalicules.

Table 1. List of the Cell Webs identified so far

Terminal web
- Unilayered (Fig. 1*A*)
  - Non-ciliated cells; chief cell of intestinal epithelium. Epithelial cells from: gall bladder, pancreatic duct, seminal vesicle, prostate, coagulating gland, uterus, ascending and descending limbs of Henle's loop, distal convoluted and collecting tubules, lacrimal gland, sweat gland, mammary gland, epididymis, vas deferens, thyroid.
  - Ciliated cells from: bronchus, trachea, ampulla of Fallopian tube, vas deferens.
  - Cells of connective tissue origin: odontoblasts, reticulo-endothelial cells of splenic sinuses (basal plate).
- Multilayered (Fig. 1*B*)
  - Epithelial cells from: intralobular (striated) and intercalated ducts of sublingual parotid, submaxillary; intralobular duct of pancreas.
  - Ducts of orbital gland, sweat gland.
  - Bile ducts.
  - Surface cell of transitional epithelium.
- With network (Fig. 1*E*)
  - Glandular epithelial cells of pancreas, serous cells of salivary glands, zymogenic cells of gastric glands, Paneth cells; mucous cells of Brunner's gland.

Pillar-like web (Fig. 1*C*)
- Non-ciliated epithelial cells of Fallopian tube.
- Epithelial cells in stria vascularis of cochlear duct.
- Supporting cells of the organ of Corti and of the maculæ (Fig. 1*D*).
- Goblet cells of intestinal and olfactory epithelium.
- Surface mucous cells of stomach.
- 'Flame-like' ciliated cells in pancreatic duct.

Circumferential web
- Partial (Fig. 1*F*): islets of Langerhans cells, corpus luteum cells.
- Complete (Fig. 1*G*, 1*H*): parenchymal cells of liver, adrenal cortex and pituitary; sebaceous cell.

Tubular web (Fig. 1*I*)
- Spermatid.

Dispersed web tonofibrils (Fig. 1*J*)
- Epidermis.
- Stratified squamous epithelia of tongue, œsophagus, forestomach and vagina.

Contractile web myofibrils (Fig. 1*K*)
- Striated muscle fibres of skeletal and heart muscle.
- Smooth muscle fibres.
- Myoepithelial cells.

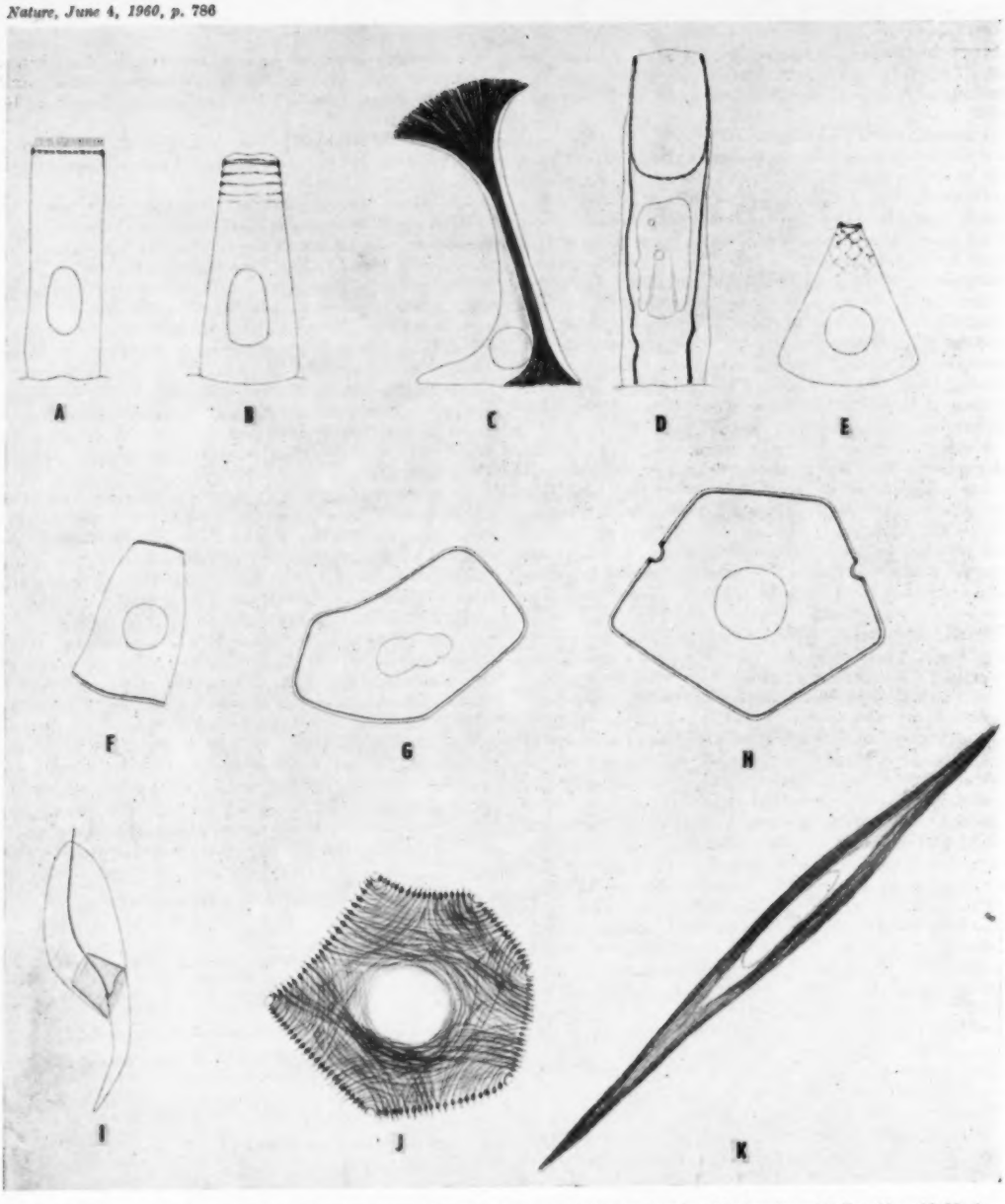

Fig. 1. Diagrammatic representation of various types of 'cell web' as revealed by the tannic acid–phosphomolybdic acid–amido black (*TPA*) technique in 2-μ sections. Cells drawn from rat tissues unless specified

A, chief cell of small intestine epithelium; a fibrous unilayered terminal web is seen at the apex of the cell immediately below the striated border. At the two ends of the web, the terminal bars are seen in cross-section

B, cell from a striated duct of a salivary gland showing a multilayered terminal web. Each layer of the cell web is connected with terminal bars

C, pillar cell of the organ of Corti in which the web filaments are packed into a dense bundle or pillar extending from the base to the apex of the cell

D, surface mucous cell of the stomach (from salamander) with a complex web containing pillar-like fibres as well as a network of fibres arranged as a basket separating the mucous droplet from the cytoplasm. Note that this cell web is attached to terminal bars

E, acinar cell of the pancreas showing a terminal web facing the lumen of acinus. The fibrous web is located between terminal bars, from which a network of filaments extends into the cytoplasm

F, beta-cell of islet of Langerhans showing a partial circumferential web lining two surfaces of the cell (above and below). These surfaces are those which face blood sinusoids

G, cell of sebaceous gland (from horse) showing a complete circumferential web under the cell membrane

H, liver cell with a complete circumferential web. The cell surface is indented in two places (right and left of diagram) so as to make up bile canalicules with the adjacent cells. The circumferential web is attached to bars running longitudinally in the walls of bile canalicules. The cell membrane lining the canalicules is subtended by a hemicylindrical web

I, in spermatids, the caudal tube (also called manchette) constitutes the web. The caudal tube is a transitory structure which appears as the nucleus of the spermatid, elongates to become sickle shaped and disappears before the cell completes its final transformation into a spermatozoon. This sleeve-like structure is located at the base of the nucleus and surrounds centrioles and flagellum

J, cell from the Malpighian layer of the skin (chestnut of the horse) showing intensely stained tonofibrils attached to desmosomes. Since the tonofibrils are distributed throughout the cytoplasm, they are considered as forming a dispersed web

K, smooth muscle fibre with well-stained myofibrils (contractile web)

This was particularly clear in parotid and external orbital glands.

So far, terminal webs have been described in simple epithelial cells. A terminal web may exist in other cell types, for example, in the odontoblast—a connective tissue cell adapted to the elaboration of dentinal matrix. In the spleen, the reticulo-endothelial cells lining the venous sinuses contained a structure described under the name 'basal plate', which extended over the entire base of the cell[15,16]. This stained with *TPA*.

Cells which were not distinctly polarized occasionally contained a *TPA*-staining fibrous layer located immediately below the cytoplasmic membrane. This structure will be referred to as circumferential web. In the cells of the islets of Langerhans, the circumferential web appeared incomplete, as it was clearly seen only at the surfaces in contact with capillaries (Fig. 1*F*). In the cells of the sebaceous glands, the circumferential web clearly extended all around the cell, thus forming a complete lining under the cell membrane (Fig. 1*G*).

The parenchymal cells of the liver also had a complete circumferential web, which reinforced bile canalicules (Fig. 1*H*). When two adjacent cells included a bile canalicule at their interface, each cell provided half the wall of the canalicule, in the form of a hemicylinder composed of cell membrane and underlying web. Junction of the two half-cylinders was ensured by longitudinal bars identical in appearance and staining properties with the terminal bars of epithelial cells, and like them, composed of a row of desmosomes.

The membranous structure described in spermatids under the name caudal tube was composed of longitudinal filaments which stained with *TPA* (Fig. 1*I*). This 'tubular web' extended caudally from the posterior region of the nucleus and enclosed in its cuff-like membrane the centrioles and the proximal part of the flagellum, both of which also stained.

The tonofibrils present in the cells of the Malpighian layers in epidermis and stratified squamous epithelia stained well with *TPA*. The bead-like structures or desmosomes which were present along the cell membranes and in which there was a confluence of the tonofibrils also stained intensely (Fig. 1*J*). Therefore, these two structures may correspond to the terminal bar-terminal web complex. The tonofibrils would thus be the web of these cells and, since they were present throughout the cytoplasm, they may be said to make up a dispersed web.

The myofibrils of striated and smooth muscle fibres (Fig. 1*K*) stained with *TPA*. In the case of cardiac muscle fibres (Fig. 1*K*), the myofibrils were seen to terminate in the intercalated disks, which were composed of intensely stained granules similar to desmosomes. Hence, myofibrils were considered to be homologous to the web of other cells and would therefore constitute a contractile web. Myoepithelial cells contained fibrils which were distinctly stained by the *TPA* technique, and would also constitute a contractile web.

Finally, the spindle fibres seen at metaphase during either mitosis or meiosis stained with *TPA*. The centrioles were also well stained, not only when observed at the two extremities of the spindle at metaphase but also at other stages of mitosis and even in resting cells. Perhaps spindle fibres and centrioles could be related to the cell web. Such relation might give significance to the suggestion that towards the end of mitosis the equatorial portion of spindle fibres gives rise to terminal bars[11].

Is it legitimate to link a variety of structures on the basis of similar staining ability? Before answering this question, it may be recalled that many years ago Zimmermann reported that structures similar to terminal bars were present in many types of cells[17]. It would therefore be reasonable to suppose that the terminal web of the intestinal epithelium also has a counterpart in many cells.

However, when the *TPA* technique was applied to various tissues in a search for structures corresponding to the terminal web, difficulties arose, as the nucleus and various cytoplasmic materials often stained to some extent. It was therefore necessary to define the terminal web as precisely as possible. In the epithelial cells of the intestine this structure exhibited three characteristic features: (1) it stained by the *TPA* technique; (2) it stained with iron hæmatoxylin and with a series of histochemical stains indicating the presence of basic proteins (somewhat different from usual basic proteins and particularly from collagen), but there was no reaction for carbohydrates, fats or nucleic acids; (3) when the terminal web was cut parallel to the plane of section, it was often possible with the *TPA* or iron hæmatoxylin techniques to distinguish fine fibrils. (Since electron micrographs of this structure showed it to be composed of fine filaments too minute to be resolved by the light microscope[12], these filaments must be arranged in bundles which appear as fibrils after *TPA* staining.)

The various types of web listed in Table 1 stained with *TPA* and tended to be fibrillar in appearance; furthermore, those that have been analysed histochemically (as will be reported elsewhere by Holde Puchtler) gave the histochemical reactions of basic proteins. Hence, it was concluded that these various forms represented different structural arrangements of the same cell organelle, for which we propose the name 'cell web'.

It has been pointed out that, when sections of intestinal epithelium were cut parallel to the cell surface, the terminal bars of the epithelial cells were not uniform rods, but rather rows of juxtaposed granules, referred to in the present work as 'desmosomes'. The desmosomes had exactly the same staining (*TPA*, iron hæmatoxylin) and histochemical properties (being composed of basic proteins) as the cell web itself. Investigation of many cell types often showed structures with the same characters as the terminal bars of the intestinal epithelium. This was true not only of the terminal bars seen in simple epithelia at one (Fig. 1*A*) or several levels (Fig. 1*B*) but also of the longitudinal bars seen along bile canalicules and intercellular canalicules in exocrine acini, and of the intercalated disks in cardiac muscle fibres. All these structures were composed of rows of desmosomes with the same properties as the individual desmosomes of the epidermal prickle cells. The fact that the histochemical properties of desmosomes were identical to those of the web itself suggested that at least part of the desmosomes would arise from aggregation of web filaments in certain areas of the cell membrane.

There were a few cells, particularly connective tissue and blood cells, in which neither web nor desmosomes were distinguishable. However, a faint *TPA* staining of fibrillar structures could be seen occasionally. For example, mature plasma cells contained a sharply staining centriole from which

fibrils radiated. Furthermore, whenever cells divided, all of them, including connective tissue cells, showed *TPA* staining of centrioles and spindle fibres, which, therefore, may be related to web filaments. Would connective tissue cells and blood cells have a web too scanty for adequate staining? If so, the web might well be present in all cells of the rat. Whatever the answer to this question may eventually be, it was clear that a large majority of the cells in this species contained a cell web. Furthermore, preliminary observations suggested that structures corresponding to the cell web were present in other mammals and in other animal groups, making up structures that in protozoa and other cells have been described as resulting from cortical or ectoplasmic differentiation[20].

What are the functions of cell web and desmosomes? *A priori*, the two main factors influencing the shape of a cell are its relation with neighbouring elements and the structural qualities of its intracellular components. Since, in the intestinal epithelium the desmosomes connect each cell with its neighbours, while the cell web functions as a support for the cell apex[1], these structures must play a part in maintaining cell shape.

Similar observations may be made in tissues other than simple epithelia. In liver cells, the desmosomes and web are located so as to account for the well-known rigidity of bile canalicules[19]. In the ear, the stiff pillars of Corti are composed of densely packed web fibrils. The tubular web (caudal tube) of the spermatids, as a result of its firmness, may be seen to push the cytoplasmic membrane aside. In contrast, the cells with little or no stainable web—leucocytes and connective tissue cells—have no definite shape. It is concluded that, while more proof of the rigidity of the web is required, perhaps by testing with microdissection needles, circumstantial evidence favours a role of this web in giving firmness and shape to the cell.

In conclusion, the cell contains a fibrillar system—for which we propose the name 'cell web'—which stains with the *TPA* technique (tannic acid, phosphomolybdic acid and amido black) and is arranged in a characteristic manner in any given cell type. In simple epithelia, the cell web predominates in the apical region of the cell, where it is arranged in single or multiple layers of fibrils, which join the granules composing the terminal bars. (These granules are referred to as desmosomes.) In many parenchymal cells—liver, adrenal, etc.—the web makes up a complete lining under the cell membrane and its fibrils enter desmosomes, which build up structures similar to terminal bars. In stratified epithelia, the web is believed to consist of the well-known tonofibrils which enter individual desmosomes, and in muscular tissues, of the myofibrils which, in cardiac muscles at least, join the desmosomes of intercalated disks. A cell web has been seen in many other cell types (for example, spermatids and reticulo-endothelial cells of spleen). It is suggested that the fibrils of the cell web are endowed with a fair degree of firmness, and combine with the desmosomes in shaping the cell.

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DETECTION AND ESTIMATION OF A FACIAL ECZEMA TOXIN, SPORIDESMIN

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THE disease of sheep known in New Zealand as facial eczema is now believed to be due to the presence on the pasture of a fungus, *Sporidesmium bakeri*[1]. The hepatotoxic principle has been isolated and crystallized[2]. Following the results of Synge and White, spots of the toxin were chromatographed on paper in water solvent and sprayed with sulphur-reacting reagents. Of those tried, only the azide–iodine reagent[3] showed a positive reaction. This reagent has now been developed in two ways—as a spray for chromatograms, and as a titration reagent for the toxin.

Run in a descending water solvent, the toxin appears to streak, possibly due to adsorption on the paper, and this may be even more pronounced if the applied spot is an impure extract. Consequently a variety of different solvent systems was tried, and finally the method adopted was to run a descending chromatogram on Whatman No. 4 paper, using methanol/water (50/50) as a solvent. This system does not entirely preclude streaking of the toxin spot, but it does reduce streaking when crude extracts are chromatographed. An azide-iodine reaction with lowered iodine content (0·005 *N* iodine in 0·1 *N* potassium iodide, containing 1·5 per cent sodium azide) has been found most satisfactory for detection of the spot. The paper is dried and sprayed with this reagent, when the toxin appears as a white spot on a brown background. After a few minutes the paper is oversprayed with 1 per cent aqueous starch solution, which reveals the white spots against a blue background, slowly fading to a permanent brown colour.

When running several chromatograms of toxic extracts in this manner it appeared that there was some relationship between the amount of toxin present and the size of the spot on the chromatogram. A standard solution of sporidesmin (with carbon tetrachloride of crystallization) in alcohol was prepared. Chromatograms of this were run, with levels of toxin applied at the origin ranging from 0·25 μgm. to 5 μgm., and over this range there was a distinct increase of spot size with the amounts present.

Using this chromatogram as a standard it has been possible to estimate approximately the amount of toxin present in a sample by visual comparison of the spot size using known amounts of toxin on the same paper. The R_F of the pure toxin is 0·88, while the spots appearing in samples are usually of lower R_F 0·81, and sometimes streaking. It is sometimes necessary to run a preliminary chromatogram with an unknown sample to get some idea of the percentage of toxin present, as the toxin spot must be within the range of the standards.

It was considered that catalysis of the iodine–azide reaction by the toxin might be utilized as the basis of a titration method for estimation of the toxin. Reagents were prepared of 0·01 *N* iodine in potassium iodide solution containing 3 per cent sodium azide[4] and 0·01 *N* sodium arsenite. The sodium arsenite solution was used in a Conway microburette. Samples of the pure toxin in alcohol solution were put into small dishes, evaporated and excess iodine–azide solution added. The dish was sealed with a ground-glass lid, and after 5 min. the unreacting iodine was titrated with sodium arsenite, using starch indicator. The titrations were carried out in duplicate, and blanks always titrated. Using 0·5 ml. of azide–iodine reagent there was a straight-line relationship between the amount of toxin added and the iodine consumed by the azide–iodine reaction, within the range up to 5 μgm. toxin. When higher amounts of toxin are required to be titrated, greater excess of the iodine–azide solution is required. Titration carried out with iodine solution without azide present shows that uptake of iodine by the toxin is insignificant under these conditions.

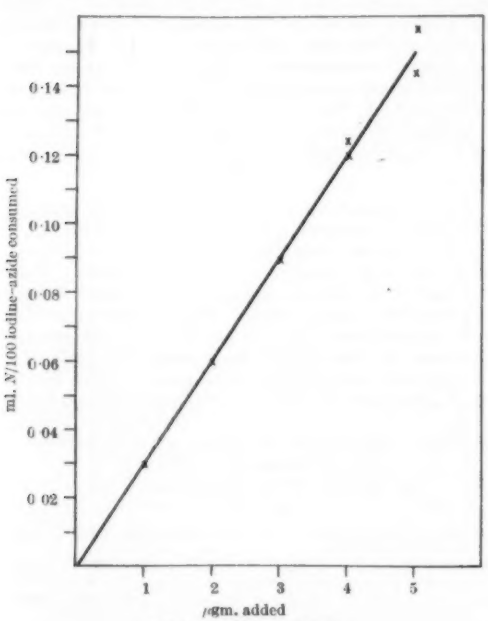

Fig. 1. Titration of toxin

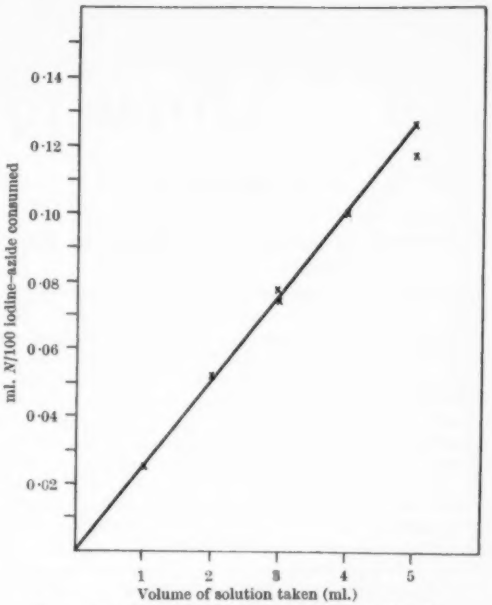

Fig. 2. Titration of spore extract (10 mgm./ml. ethanol)

Methanol extracts of spores at the petroleum ether wash stage of purification[3] were made up in alcohol solution and the insoluble material allowed to settle out. Aliquots of this solution were titrated as above, and the iodine consumption appeared to be proportional to the amount of extract added. It therefore appears that even in these crude extracts, a measure of the iodine consumption by the azide–iodine reaction may be used to estimate the amount of toxin present. The following is a typical example of a toxic extract of spores; 10 mgm. of the extract at the petroleum ether wash stage was made up to 1 ml. in alcohol. Chromatography of aliquots of this solution showed that the only azide–iodine reacting spots present were due to the toxin, and it was estimated by the comparative method that the extract is 4 per cent toxin.

Titration of a series of aliquots of this solution, using information from Figs. 1 and 2, indicates that the extract is 4·2 per cent toxin. This result is in good agreement with the level of toxicity obtained by biological tests, carried out by colleagues in these laboratories (based on production of keratitis of rabbit's eye, injury to HeLa cells and characteristic biliary lesions produced in the liver of rabbits).

The results of this suggests that the method so far used may be applicable to the detection and estimation of the toxin in many media, and work is in progress to estimate toxin in pasture.

A more complete study of the catalysis of the azide–iodine reaction by the toxin will be made in the future.

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LETTERS TO THE EDITORS

MATHEMATICS

Analysis of Accelerated Motion in the Theory of Relativity

Conventional treatments of accelerated motion in the theory of relativity have led to certain difficulties of interpretation. Thus, Crampin, McCrea and McNally[1] mention the lack of uniformity in the correspondence of events as depicted by the transformation of Born and Biem. Again, Donahue and Leffert[2] and Moller[3] discuss certain reversals in the apparent gravitational field of an accelerated body. I have found[4] that these difficulties may be avoided by simpler analysis based on the use of restricted conformal transformations. In the conformal theory the velocity of light remains constant even for experimenters in accelerated motion.

The problem considered is that of rectilinear motion with a variable velocity v. I introduce two co-ordinate systems, A, (x,t) and B, (x',t'). The motion takes place along the x or x' axis.

The correspondence between the xt and $x't'$ systems may be expressed quite simply by the transformation:

$$\begin{aligned} x' + t' &= F(x + t) \\ x' - t' &= G(x - t) \end{aligned} \qquad (1)$$

The velocity is given by:

$$v = \frac{g - f}{g + f} \qquad (2)$$

Here f and g are the derivatives of F and G with respect to their arguments $x \pm t$. I now suppose that A, (x,t) is an inertial system, and in order to satisfy the relation of equivalent scale in the vicinity of B, I apply the boundary condition:

$$fg = 1 \text{ along } x' = 0 \qquad (3)$$

If the motion of B is given then this relation, together with equation (2), is sufficient to determine the functions F and G. As determined in this way, this transformation becomes tangent to instantaneous Lorentz transformations all along the path of B and in the case of uniform velocity reduces to the Lorentz transformation everywhere.

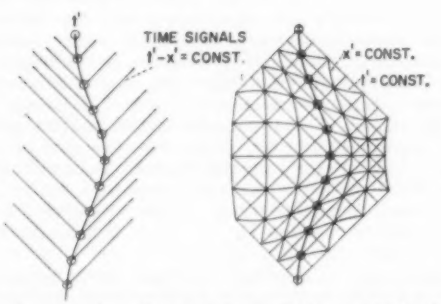

Fig. 1. A system of conformal co-ordinates associated with non-uniform motion

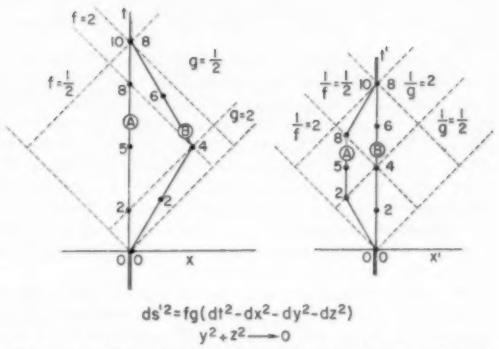

Fig. 2. Example showing application of restricted conformal transformations

Fig. 1 shows how such $x't'$ co-ordinates may be constructed graphically with the aid of periodic time signals originating in the B system. Such signals generate a family of outgoing waves, represented by the characteristic lines $t \pm x =$ constant. If these lines are now extended backward, a corresponding family of incoming signals will be represented. Intersections of the characteristic lines may then be identified with the events of synchronization of the various clocks of the B system.

Extension of the theory of relativity by conformal transformations in four dimensions was considered many years ago by Bateman[5]. It seems that the group $C4$ admits only restricted motions, and of these the Lorentz transformation alone maintains equality in the scale relation. Therefore, we do not speak of a conformal transformation of the whole space. However, by restricting attention to a narrow cylindrical region around the x-axis, conformal mappings can be employed locally, so that:

$$ds'^2 = fg(dt^2 - dx^2 - dy^2 - dz^2) \qquad (4)$$

for $y^2 + z^2 \to 0$.

Fig. 2 illustrates a simple example of the type discussed in connexion with the clock paradox. At $t = 0$, B starts away from A at the velocity 3/5. At $t = 5$, B reverses its motion and returns. Values of f and g in various regions are indicated between characteristic signal lines. The condition of local scale equivalence, $fg = 1$, results in a 20 per cent reduction of the elapsed time along the path of B. In addition to the time discrepancy, there appears also a discrepancy in the relative spatial displacements.

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GEOPHYSICS

Anisotropy of Susceptibility and the Natural Remanent Magnetization of Some Welsh Slates

In order to investigate the effect of the deformation associated with mountain building upon the anisotropy of susceptibility and the natural remanent magnetization of rocks, approximately a hundred specimens were prepared for measurement from some fifty samples of Lower Palæozoic Welsh slates cleaved during the Caledonian orogeny. The anisotropy in fields of less than two œrsted was determined with a transformer bridge[1] and the natural remanent magnetization with an astatic magnetometer. The results showed that the cleavage is a plane of high susceptibility, and that the remanent vector is parallel to it. In some of the slates it appears that the direction of natural remanent magnetization has been controlled by the anisotropic susceptibility, and in others that the pattern of anisotropy and the natural remanent magnetization have been aligned with the cleavage independently. I am indebted to Dr. J. C. Belshé and Dr. R. W. Girdler, with whose help the susceptibility work was begun.

It was only possible to measure the direction of natural remanent magnetization of the specimens from four sites, the remainder being too weakly magnetized. Without exception the vectors lay close to the cleavage plane; fifty of the fifty-six measured were within 20° of it and twenty-six within 5°. Figs. 1*a* and 1*b* are equal-area projections showing the direction of natural remanent magnetization relative to the field orientation of the rocks. In the first plot (Fig. 1*a*) the mean directions of natural remanent magnetization of the specimens from three of the sites have been joined by great circles to their respective cleavage planes. The scatter about these mean directions is small, for example, the radius of the cone of 90 per cent probability (α), and the Fisher precision factor (k)[2] were 8° and 29 respectively for the Dolgelley slates. The mean directions are related to the cleavage and therefore distinct for each site, but they are nevertheless in the same general direction as previous workers have found in the nearest rocks of Devonian age[3]. The natural remanent magnetization of these slates is due to pyrrhotite. In contrast, the remanent vectors of the Bethesda slates (Fig. 1*b*) have neither a small scatter nor directions comparable to any suggested for British rocks. Their magnetization is due to hæmatite. The remanence of the Bethesda slates is almost unaffected by alternating-current demagnetization up to fields of about 400 œrsted, while the magnetization of the pyrrhotite-controlled specimens is wiped out by fields of 250 œrsted.

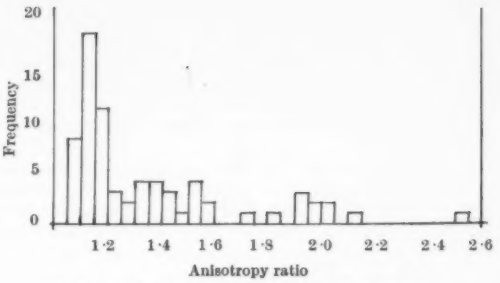

Fig. 2. Histogram of anisotropy ratios of Welsh slates

Fig. 2 is a histogram of the anisotropy ratios found in the slates. Defining the P factor as the ratio of maximum to minimum susceptibility, we see that no P factor of less than 1·05 was observed while values between 1·1 and 1·2 were the most common. The ferromagnetic mineral in those specimens with P factors greater than 1·4 was shown by X-ray analysis and Curie-point determination to be invariably pyrrhotite. The anisotropy of the Welsh slates exceeded that of the few uncleaved sedimentary and igneous rocks which were collected alongside them for comparison.

To consider the relation between the pattern of anisotropy and the cleavage, we now compare the direction of minimum susceptibility and the pole to the cleavage plane (Fig. 3). The similarity of these two directions demonstrates that the maximum intermediate plane of the susceptibility magnitude ellipsoid is approximately parallel to the cleavage plane. The anisotropy of the slates will be due either to shape effect or to crystalline anisotropy of the ferromagnetic minerals. If it is caused by the former then there will be preferred orientations of the long axes of grains of intrinsically isotropic susceptibility. If it is caused by the latter the ferromagnetic grains must have preferred crystallographic orientations. Either possibility is easy to imagine in a slate and both are observable in various minerals, particularly the micas.

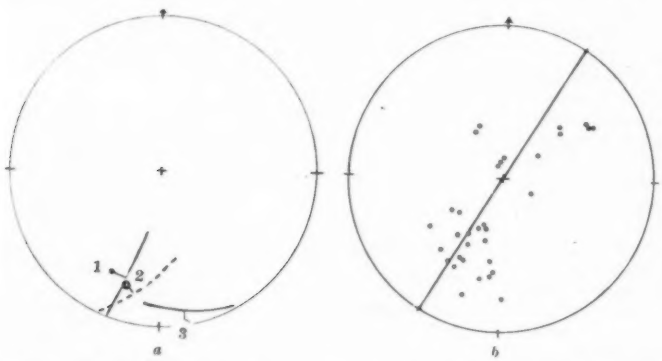

Fig. 1. The direction of natural remanent magnetization and the orientation of the cleavage. (*a*) Slates from Barmouth (1), Talsarnau (2) and Dolgelley (3). (*b*) Cambrian slates from Bethesda. Upper hemisphere poles and great circles, ○/; lower hemisphere poles and great circles, ●/

In reflected light the pyrrhotite in the markedly anisotropic slates can be seen to be in the form of platey grains lying parallel to the cleavage plane. However, the anisotropy of the slates is not likely to be due to shape because the susceptibility of the pyrrhotite is too small for the effect of the anisotropic demagnetizing field to be observed[4]; powders of this pyrrhotite have a susceptibility of 8×10^{-3} e.m.u./cm.^{3}. Since pyrrhotite is known to have an easy plane of magnetization and a difficult direction normal to the plane, the observed anisotropy of the slates

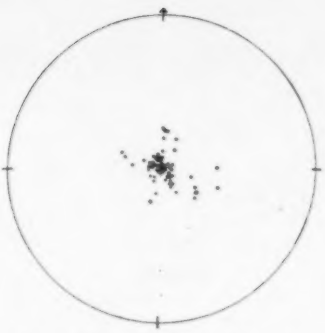

Fig. 3. Equal area projection of the minimum susceptibility directions with the cleavage plane horizontal. Pole to the cleavage, ⊕

could be due to an alignment of the high-susceptibility basal planes parallel to the cleavage. This explanation was confirmed by further examination of the optically uniaxial pyrrhotite in reflected light. In sections cut parallel to the cleavage the grains are predominantly almost isotropic, while in sections perpendicular to cleavage they invariably show distinct anisotropism and moreover they extinguish parallel to the plane of the plates. Hence the platey grains represent the common tabular habit of pyrrhotite, and therefore there is an alignment of the basal planes parallel to the cleavage and this causes the slates to be magnetically anisotropic.

The thermo-remanent magnetization of anisotropic substances has been shown to be deflected from the ambient external field towards the direction of high susceptibility[4,5]. Three specimens of the Crogenon slates, which have pyrrhotite, were given a thermo-remanent magnetization and a mean deviation of 30° recorded. Since the Curie-point of pyrrhotite is 320° C., which may have been exceeded during the metamorphism associated with the Caledonian orogeny, it is possible that the natural remanent magnetization had a thermal origin and is parallel to a component of the Devonian geomagnetic field in the basal planes of the pyrrhotite. Such a mechanism of magnetization does not account for the direction of the natural remanent magnetization of the Bethesda slates because their anisotropy is too small to produce the necessary deviation from the ambient field. Thus we must look to some more direct effect of the stress environment which produced the cleavage.

The magnetic susceptibility of slates is then distinctly anisotropic. The pattern of anisotropy and the direction of natural remanent magnetization are related to the cleavage and were, therefore, determined either directly or indirectly by the stress environment during metamorphism. It is important to note the possible indirect effect of stress demonstrated, for example, by the anisotropic Ballachulish slates in which it is known[6] that the pyrrhotite formed after the slates were cleaved. The mere presence of a foliation such as a cleavage or bedding plane can cause anisotropy if secondary pyrrhotite forms. The effect of anisotropic susceptibility upon magnetization is sufficient to account for the direction of natural remanent magnetization in the slates with pyrrhotite, but not of those with hæmatite. Thus it has been possible to explain the nature of the anisotropic susceptibility of some of the slates in terms of recrystallization controlled by past stress environments and it should not be difficult to extend this work. However, the nature of the remanent magnetization and the means by which the stress controls its direction are still uncertain.

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PHYSICS

Structure of High-Rank Coals deduced from Helium Densities

Wandless and Macrae[1] were the first to show that a relationship exists between the true density of coals and their hydrogen content. Franklin[2] extended this work, and on plotting the specific volume ($1/d$) of a number of coals found that an extrapolation of the straight line drawn through the points, to zero hydrogen content, did not pass through the point for graphite (Fig. 1). For this reason she proposed the hypothesis that the structure of coals is "markedly different from that of graphite". This is in disagreement with the generally accepted theory that coal molecules are basically fused, polynuclear aromatic

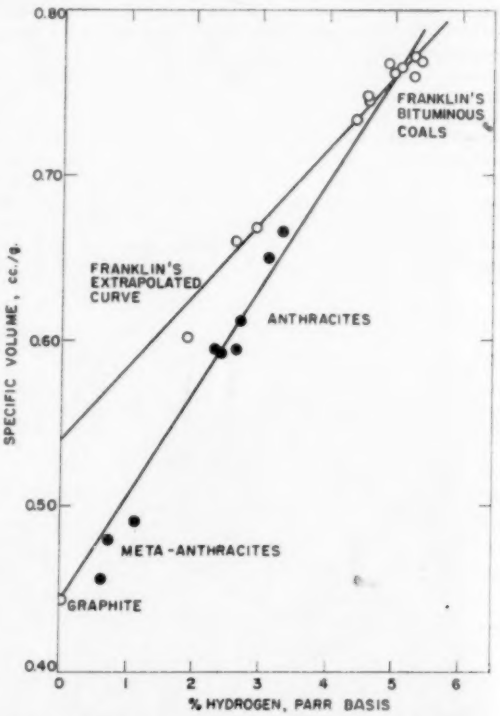

Fig. 1. Specific volume of coals versus percentage hydrogen

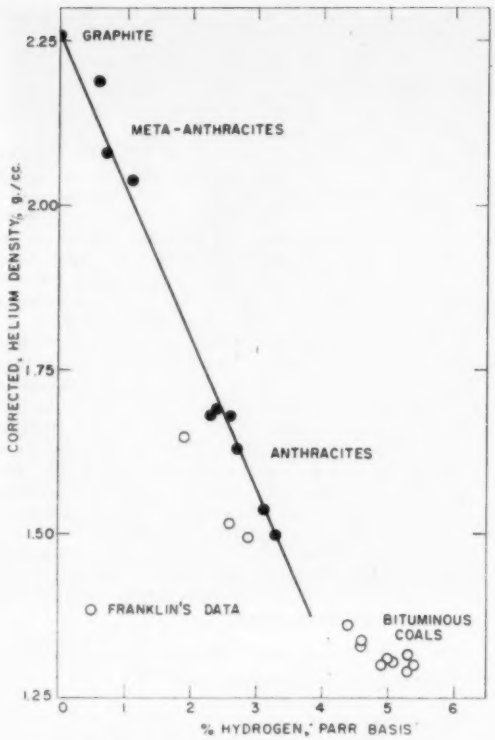

Fig. 2. Density of coals versus percentage hydrogen

structures, that is, islets of small graphitic monolayers which may or may not be associated vertically, and to which various aliphatic groupings are attached. This is suggested in van Krevelen and Chermin's[3] plot of density of coals versus carbon content, in which the density of graphite is considered to be "the true limit" of coalification.

If coals, especially those of high rank, are composed of molecules which are basically related to graphite, then the hydrogen atoms present in these coals should decrease the density of the coals in proportion to the amount of hydrogen present. To test this hypothesis, we have determined the helium densities of three meta-anthracites and six anthracites (Table 1). These were corrected for mineral matter by the method of Wandless and Macrae[1] and the corrected densities plotted against hydrogen content in Fig. 2. The specific volumes of these coals are also plotted in Fig. 1. It will be observed that density gives a closer correlation than specific volume for the high-rank coals. On the other hand, specific volume gives a fair correlation that extends into the bituminous coal region. In either case, it will be observed that graphite constitutes the end-point of these plots, and that there is no reason to assume that high-rank coals have a carbon structure basically different from that of graphite.

Table 1. Helium Densities and Analyses of Coals

Coals	Analysis, Parr basis % H	% C	% S	Helium density (gm./c.c.) d, uncorr.	d, corr.*
Meta-Anthracites					
Rhode Island No. 1	0·6	97·1	0·47	2·330	2·08
No. 2	0·7	97·6	0·08	2·191	2·19
Michigan†	1·1	96·5	0·30	2·100	2·04
Anthracites, Penna.					
Eastern Middle,					
No. 1	2·3	95·5	0·46	1·722	1·68
No. 2	2·4	93·7	0·48	1·753	1·69
Western Middle,					
No. 1	2·6	93·9	0·45	1·824	1·68
No. 2	3·1	92·7	0·66	1·609	1·54
Northern Field	2·7	92·9	0·56	1·664	1·63
West Southern Field	3·3	93·2	0·78	1·542	1·50

*Corrected for mineral matter following Wandless and Macrae (ref. 1).

† Pre-Cambrian algal coal, reputed to be the oldest known coal in the world.

The equation for the line drawn through the densities of the high-rank coals in Fig. 2 is $y = 2{\cdot}26 - 0{\cdot}23x$, where y is density and x is the percentage hydrogen. As will be observed in Fig. 2 this correlation must not be extended too far down the coal series. Coals having hydrogen contents greater than about 3·5 per cent tend to have densities greater than the equation predicts. No doubt this is due to the increasing amount of oxygen and other atoms introduced along with hydrogen into the lower rank coals, and on this basis the density of these coals should not be expected to correlate with their hydrogen content as the only variable affecting density.

A further test of the reliability of the equation above can be made with three high-rank coals investigated by Dulhunty and Penrose[4]. The hydrogen contents of these coals are 2·0, 3·1 and 3·6 per cent. These values give calculated densities of 1·800, 1·547 and 1·432 respectively, which compare favourably with the observed densities of 1·804, 1·512 and 1·430 in the same order.

Fairly good densities of coals so far as the bituminous rank may be calculated using the equation for the line drawn through the specific volumes of the coals in Fig. 1. The equation for this line is $y = 0{\cdot}442 + 0{\cdot}064x$; where y is the specific volume and x is the percentage hydrogen. By calculating the specific volume of a coal with this equation, the density is easily obtained as the reciprocal.

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Effect of Carrier Gas on the Sensitivity of Thermal Conductivity Detectors

The sensitivity of thermal conductivity detectors is proportional to the variation of the wire temperature (t) with the volume fraction (x) of the vapour in the carrier gas. Ray[1] has shown that for small values of x:

$$dt/dx = a(k_1 - k_2)/k_1^2 \qquad (1)$$

where k_1 and k_2 are the thermal conductivities of carrier gas and vapour, respectively, and a is a constant depending on the geometry of the detector cell and the square of the wire current. This equation,

and the conclusion drawn by Ray from it, has led to a discussion[2-6] of the merits of carrier gases of high and low thermal conductivity with respect to the sensitivity of thermal conductivity detectors.

It should be noted, however, that Ray's equation is valid only when the thermal conductivity of the mixture satisfies the linear relation $k_m = (1 - x)\,k_1 + xk_2$. In general, thermal conductivities of mixtures are not a linear function of the composition, although quite often, at high dilutions, a linear relation can be obtained by substitution of an effective k_2 for k_2 (ref. 7). However, the value of this effective k_2 depends on the choice of the carrier gas.

It is also possible to replace the linear k_m versus x relation by a more widely applicable empirical equation[8]. This would lead to very complicated formulæ. Instead, we will consider another extreme relation, for example, $1/k_m = (1 - x)/k_1 + x/k_2$ (refs. 9 and 10). Application of this equation gives:

$$dt/dx \simeq a(k_1 - k_2)/k_1k_2 \qquad (2)$$

In contrast with equation 1, equation 2 predicts a higher sensitivity of the detector for carrier gases of high thermal conductivity.

Table 1. Experimental and Calculated Detector Sensitivities (millivolts per volume fraction) for Methane in Hydrogen and Nitrogen ($x \sim 0{\cdot}025$)

i (m.amp.)	Hydrogen			Nitrogen		
	Exp.	Eq. (1)	Eq. (2)	Exp.	Eq. (1)	Eq. (2)
30				0·28	0·3	0·2
60				2·25	2·5	2
90				8·6	8·5	6
100	11	4	22	12	12	9
120				21	21	15
150				53	45	35
200	91	33	175			
250	180	65	350	372	265	195
400	790	275	1,430			

Thermal conductivities at the average cell temperature were taken from Berl, W. G., "Physical Methods in Chemical Analysis", 2 (New York, 1931). The temperature was calculated by using the equation for the heat balance in the detector cell.

Table 2. Experimental and Calculated Detector Sensitivities for Propane in Hydrogen and Nitrogen ($x \sim 0{\cdot}025$)

i (m.amp.)	Hydrogen			Nitrogen		
	Exp.	Eq. (1)	Eq. (2)	Exp.	Eq. (1)	Eq. (2)
30				0·42	0·3	0·4
60				4·4	2·5	3·5
90				15·4	8	11·5
100	20·8	4·5	45	23	11	15
120				37	18	25
150	75	15	155	69	35	45
200	176	35	375	154	75	100
225				195	100	140
250	340	70	730	207	125	165
275				207	150	200
300	564	125	1,260	142	180	220
400	1,390	295	2,950			

Tables 1 and 2 show some experimental sensitivity values, obtained with a four-wire thermal conductivity detector (wire thickness 0·003 cm.; wire length 12 cm.; wire resistance at 0° C. 16·3 ohm; temperature coefficient of the platinum wires 0·0037; detector temperature 25° C.).

The sensitivities (E/x) are expressed in mV. per volume fraction of the vapour. In addition to the experimental values, sensitivities were calculated using equations 1 and 2. A direct comparison between the experimental and calculated values can be made when dt/dx is converted into dE/dx by means of an approximate equation for the out-of-balance voltage of the bridge,

$$dE/dx = \tfrac{1}{2}\, i\, R_0\alpha\, dt/dx \text{ (ref. 7)}$$

where i is the total bridge current, R_0 the resistance of the filaments at 0° C. and α the temperature coefficient of these resistances.

Up to a total bridge current of 250–300 m.amp. (the current should not exceed this figure when nitrogen is used as a carrier gas), the system methane – nitrogen gives higher sensitivities than the system methane – hydrogen, while for propane higher sensitivities are obtained when hydrogen is used as a carrier gas. It is interesting to note that for the propane – nitrogen system the sensitivity reaches a maximum at a current of about 250 m.amp.

The agreement between the experimental values and the sensitivities calculated with both (1) and (2) is rather poor. In our opinion this clearly illustrates the difficulty of predicting sensitivities from k_1 and k_2 without knowing the exact value of the thermal conductivity of the mixture. Although in most cases a calculation of sensitivities will be impossible, our experiments confirm the view[2-6] that the highest sensitivity values can be obtained by employing a carrier gas of high thermal conductivity.

Note added in proof. A. B. Littlewood, in a recent communication (*Nature*, **184**, 1631; 1959), gives a semi-empirical method of calculating sensitivities from molecular dimensions.

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METALLURGY

Reaction between Copper and Solid Oxygen condensed at Very Low Temperatures from a Gas Discharge

In recent years numerous studies[1] have been carried out on the short-lived reactive species formed in an electric discharge after trapping and storing them on a low-temperature surface. This communication is concerned with the interaction between the discharge products of oxygen and the metal surface upon which they have been trapped at a low temperature. Copper was chosen as the metal to study because it is frequently used as a condensing surface. Also some data are available on the low-temperature (77° K.) oxidation of copper by molecular oxygen[2,3] and on the reaction of oxygen discharge products with copper at elevated temperatures[4].

Both single crystal and polycrystalline specimens of 99·99 per cent copper were studied. Their surfaces were specially prepared so that they were clean,

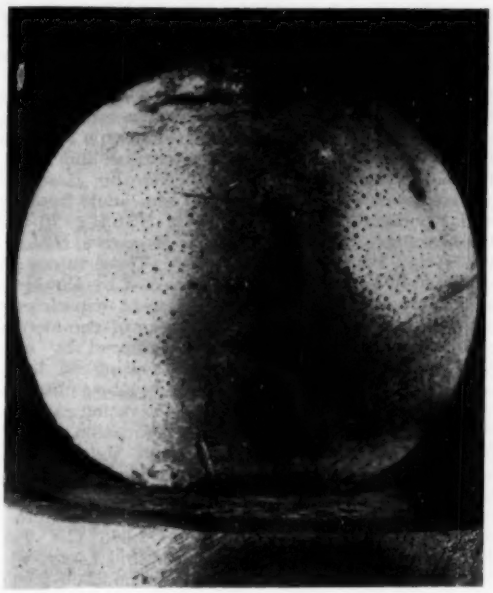

Fig. 1

strain-free, and smooth[5]. Some preliminary experiments were carried out in which a single-crystal sphere at 4·2° K. was placed in the path of a stream of ordinary tank oxygen which had been passed through a 2,450-Mc. electrodeless discharge to dissociate it partially. Small specks of a reaction product could be detected visually (Fig. 1). These were later identified as cupric nitrate by X-ray diffraction, indicating that the tank oxygen contained small amounts of nitrogen. The random distribution of the reaction product on the surface of the spherical single crystal rules out orientation effects. When oxygen of 99·998 per cent purity was used, no reaction could be detected visually.

Most of the experiments were carried out in an apparatus in which the copper specimen, after annealing in hydrogen at 450° C., could be cooled to a desired temperature (usually 4·2° K.) and the thickness of films formed on its surface measured using an ellipsometer[6].

Oxygen was deposited on the bare metal surface, and the thickness of the gas film was measured with the ellipsometer. When discharge products were desired, the oxygen stream was first passed through the microwave generator wave-guide before impinging on the metal surface. After warm-up of the copper specimen to room temperature, ellipsometer readings were again taken.

It was found that, under these particular experimental conditions (Dewar pressure during deposition, about 2×10^{-5} mm. mercury; inlet pressure of oxygen, 0·15–0·7 mm. mercury), molecular oxygen had no effect at any temperature up to 300° K. When the pure oxygen was passed through the microwave discharge, however, a film of reaction product of about 20 Å. thickness formed if the copper surface was at 4·2° K. during deposition. The films formed on all surfaces studied ({100}, {110}, and {111}) were of the same thickness, and this thickness was not increased by subsequent runs. Deposition of extraneous material was ruled out by the fact that no film was found on warm-up on an adjacent gold surface employed as a control. The discharged oxygen had no effect on copper at 40° K. or higher. Further, if a thin film (400 Å.) of molecular oxygen was placed on the copper surface at 4·2° K. and discharged oxygen condensed on top of this, no reaction was observed. Finally, since ozone is formed as one of the discharge products, a check was made to see if it was responsible for the reaction observed by depositing pure ozone on the copper at 4·2° K. As with molecular oxygen, no reaction was observed.

It appeared, then, that a reaction took place on the surface between 4° K. and 40° K., forming a product that is solid at room temperature. The species responsible for the reaction observed is probably atomic oxygen, and the reaction product, an oxide.

The concentration of the trapped oxygen atoms is very low, probably of the order of tenths of a mole per cent[7]. Most of these would recombine at a temperature between 4·2° and 40° K., where they have sufficient mobility, rather than react with the metal surface. The formation of an oxide film may be due to a reaction between the condensed molecular oxygen and the copper surface. The activation energy for this reaction could be provided by the local heating produced by recombinations of oxygen atoms. Further work is in progress on the system described here and on other metals and gases.

We wish to acknowledge the assistance of Dr. G. J. Minkoff of the Imperial College of Science and Technology, London, in carrying out the preliminary experiments while a guest worker at the National Bureau of Standards.

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ENGINEERING

High-Temperature Creep of Graphite

Davidson and Losty[1] have proposed a logarithmic equation to describe the creep behaviour of synthetic graphites in the temperature-range 1,300–2,000° C. Analysis of results obtained at this laboratory by Martens, Jaffe and Button[2,3] indicates that this equation also gives a good description of graphite creep at higher temperatures, up to 3,000° C. However, there are appreciable differences in the relation of creep to temperature and stress in the two temperature ranges.

Short-time tensile creep data in the temperature-range 2,400–3,000° C. have been obtained for several commercial grades of synthetic graphite using test specimens with a 0·25-in. diameter by 1-in. long gauge section. Heating was accomplished in an automatically controlled graphite tube furnace in a helium

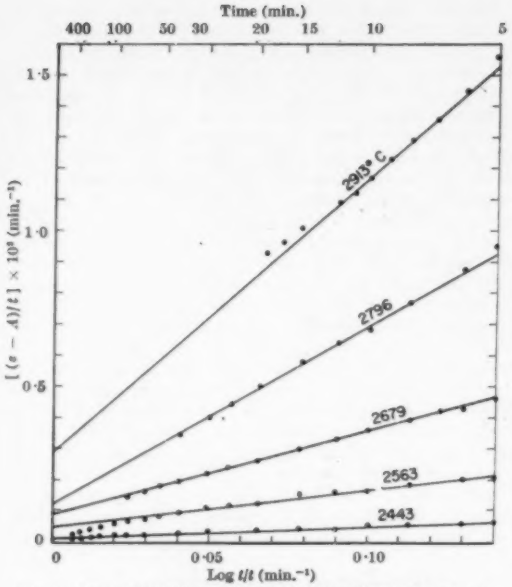

Fig. 1. Creep of *H3LM* graphite plotted according to equation 1. Stress 2,000ψ, parallel to the grain

atmosphere. Tests were generally run to fracture, or 400 min., whichever occurred first. The results discussed here were obtained on specimens cut from one block each of Great Lakes Carbon Co. *H3LM* and Speer Carbon Co. 3499. (The data analysed here were obtained from blocks designated 0401 and 0106 in refs. 2 and 3.) All tests were made on specimens as machined without any preliminary heat treatment, unless otherwise noted. Further details of the creep measurement techniques have been described elsewhere[2,3].

The equation found by Davidson and Losty is of the form :

$$\varepsilon = A + B \log t + Ct \qquad (1)$$

Here, ε is the creep strain, t is time, and A, B and C are constants for each test. Some representative results, plotted on co-ordinates which give a straight line if equation 1 is obeyed, are shown in Fig. 1. The results plotted are for times greater than 5 min. It is evident that the fit is quite good. The points tend to fall above the best straight line at short times and below it at long times. The short-time deviations, which were observed in all the results, may arise in part from errors in the estimation of the A values and from strain measurement errors. The deviations from linearity at long times (for example, the 2,563° C. curve, Fig. 1) cannot be ascribed to these errors. Steady-state creep-rates (C values) determined from the final portion of the creep curves agree with those determined by drawing a tangent to the elongation versus time curve, whereas rates obtained from a linear fit to the early part of the curve (as in Fig. 1) are higher. This behaviour was characteristic of the *H3LM* graphite, but was not generally observed in the 3499 material. This phenomenon apparently reflects a change in the condition of the material which is brought about by the creep deformation. The nature of this change is not understood.

The constants B and C were both found to be Arrhenius functions of the temperature :

$$B = B_0 \exp(-\Delta H_B/RT) \qquad (2a)$$

$$C = C_0 \exp(-\Delta H_C/RT) \qquad (2b)$$

where B_0 and C_0 are functions of the applied stress, R is the gas constant, T is the absolute temperature, and ΔH_B and ΔH_C are observed activation energies. This agrees with the results of Davidson and Losty. The activation energies obtained here for B and C, in that order, were : 112 and 106 kcal./mole from the initial portion of the creep curve, *H3LM* ; 70 and 180 kcal./mole for the final part of the curve, *H3LM* ; and 81 and 204 kcal./mole for 3499. These values are appreciably higher than those obtained by Davidson and Losty (20–50 kcal./mole). Recent experiments have indicated that, for some blocks of commercial graphite, the creep behaviour is altered by heat treatment at 2,800° C. prior to testing at lower temperatures. Therefore, the energy-values obtained here are best regarded as apparent activation energies. Preliminary results indicate that equation 1 still holds following heat treatment, but the observed activation energies are somewhat lower for some blocks. Both B and C were found to be approximately proportional to the square of the initial applied creep stress over the range 600–3,100ψ at 2,565° C. This differs from the results of Davidson and Losty, who found a linear dependence on stress in the 1,300–2,000° C. range.

Some observations were also made on the creep recovery phenomena. In these experiments, the load was removed after a total creep strain of 5–20 per cent and the strain recovery observed at the same temperature as the creep. In some cases the temperature was increased for periods of ½–1 hr. during recovery. This accelerated the rate of recovery but did not increase significantly the total amount of recovered strain. The amount of recoverable strain ranged from one-tenth to one-third of the total creep elongation. For both the as-received and the pre-annealed specimens, the recoverable strain was generally less than one-half of the transient creep strain (the $B \log t$ term in equation 1). This contrasts with the results of Davidson and Losty, who found that the transient creep strain was completely recoverable. The larger total creep strains employed in the present investigation may contribute to this difference in behaviour.

A sigmoidal curve is obtained when the strain recovery is plotted against the logarithm of the time after removal of the load. A description in terms of a superposition of relaxation processes with a broad spectrum of relaxation times is indicated to account for the curvature in this plot at very short and very long times. However, the recovery behaviour can be approximated by a linear logarithmic time dependence, consistent with the use of equation 1 to describe the creep behaviour. Such a description deviates significantly from the observed results only at very short and very long times (for example, < 2 min. and > 200 min. at 2,680° C.).

Equation 1 is compared with two other creep equations, applied to the analysis of high-temperature creep of graphite, in a paper published elsewhere[3].

Grateful acknowledgment is made to L. D. Jaffe and H. E. Martens for helpful discussions and to King Titus for assistance with the computations.

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CRYSTALLOGRAPHY

Mosaic Texture of Graphites

Crystal sizes in graphitic carbons are commonly in the μ or sub-μ range. The average dimensions of the crystallites (that is, the parallel-layer groups of condensed aromatic ring networks) in any particular material are usually deduced from measurements of the widths of X-ray powder diffraction maxima. Although the measurements are complicated by the presence of stacking disorders, techniques exist[1] for eliminating the effects due to these. The dimensions thus obtained are: *La* the average diameter of layer-network over which there is considerable perfection, *t* the average height of parallel-layer group over which the stacking is fully ordered, *Lc* the total height of the group irrespective of the presence of disordered (turbostratic) stacking. With crystallite dimensions greater than about 200 Å. the accuracy of the determination depends very considerably, and to an extent increasing rapidly with crystal size, on the corrections for extraneous (instrumental, absorption, etc.) line-broadening effects. Nevertheless, for good natural graphites the crystallite dimensions are consistently found to be not more than a few thousand Å. (ref. 2 and Steward, E. G., and Cook, B. P., unpublished work). For artificial graphites the dimensions are usually less than 1000 Å. (refs. 2,3,4 and Steward, E. G., and Cook, B. P., unpublished work).

Recent considerations[5] of the graphitization process in anthracites, pyrolytic and evaporated carbons suggest that current views on graphitization are unnecessarily restricted by attaching too great an ultimate and exclusive importance to dimensions measured in the above manner: the concept of larger mosaic '*M*' units has been suggested. Kuroda[6] has used electron diffraction to study cokes and has recently reported evidence of the presence of sub-structure with preferred orientation. Further evidence in support of the general concept could also be deduced from the more recent electron microscope studies by Dawson and Follett[7], who have reported crystal layer-sizes of about 3000 Å. in a graphitized coke for which X-ray diffraction had given *La* values of less than 800 Å.

Visual examination of crystal flakes of natural graphites clearly reveals a highly oriented mosaic structure. Such flakes may have dimensions of several mm., but X-ray diffraction measurement would indicate parallel-layer group dimensions much smaller than this. That the '*L*' dimensions deduced from the X-ray diffraction broadening arise from the sub-structure may be demonstrated quite simply by the X-ray diffraction Laue method. Fig. 1*a* shows

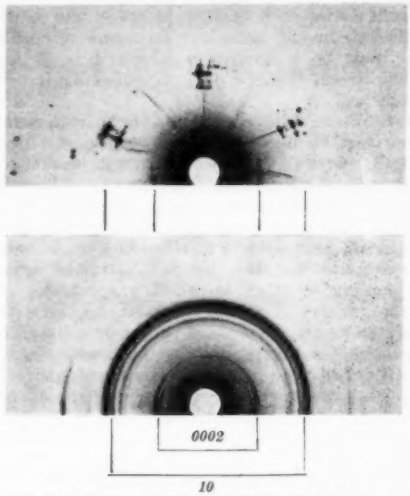

Fig. 1. Laue-type X-ray diffraction photographs of graphitic carbon flakes: 50μ beam approximately normal to flakes. (Copper radiation.) *a*, Natural (Ceylon) graphite; *b*, petroleum coke: graphitized at 2,700° C.

a Laue pattern taken with an X-ray beam of 50μ diameter, approximately normal to a flake selected from a sample of natural graphite. The pattern is seen to approximate to that of a single crystal. However, the spots show break-up typical of a mosaic crystal and it is the diffraction broadening of the individual reflexions within the spot-clusters which is dependent on the size of the mosaic sub-blocks (that is, the parallel-layer groups) and which is the diffraction broadening normally recorded in the 'powder method'. The well-modulated *10* reflexions and the sixfold symmetry of these indicate that there is little stacking disorder of layer-planes within the parallel-layer groups and that there is strong preferred orientation of the *a*-axes of the various parallel-layer groups comprising the mosaic: the weak intensity of *0002* reflexions indicates that the layer-planes are essentially perpendicular to the X-ray beam.

It is logical that a similar mosaic condition exists to different extents in graphitic carbons generally. This is illustrated in Fig. 1*b*, which shows the Laue pattern obtained with the 50μ beam, from a thin flake of graphitized coke: the selected flake was not necessarily typical of the whole coke. The intensity of the *0002* reflexion relative to that of the *10* reflexions again indicates a mosaic formed by strong preferred coplanar orientation, parallel to the surface of the flake, of the *La* areas: the pronounced arcing of the weak *0002* reflexions is due to the flake being somewhat cylindrically curved about an axis approximately normal to the beam. The extensive modulation of the *10* band into the component 3-dimensional $10\bar{1}0$ and $10\bar{1}1$ reflexions shows that turbostratic stacking is not appreciable: the circumferential arcing of spots to form smooth rings is therefore indicative of a large number of small (*La*) units and of lack of preferred orientation of the *a*-axes of these within the above mosaic planes.

These simple experiments thus demonstrate by X-ray diffraction methods the existence one would expect of mosaic units in graphitic carbons. In such a material the particles will not necessarily have a uniform $L : M$ size relationship and there will also be

different types of boundary between the L volumes comprising an M mosaic. In some circumstances there may be a condition where $L = M$ and the particle would then be an unoriented agglomerate of crystallites.

The texture will, of course, vary profoundly from one carbon to another. In a well-crystallized (for example, natural) graphite there will be considerable 3-dimensional near-parallel alignment of layer-groups to form mosaics closely approximating to single crystals. Larger misorientations between the mosaic sub-blocks, particularly in the Ma plane, would in part account for the limited ultimate crystalline development in other so-called 'graphites'.

Thus, while X-ray diffraction has the unique ability to reveal the size of the basic crystalline unit in carbons, further parameters need to be recognized when assessing the general crystalline texture of such materials and when considering their physical properties.

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Crystal Structure of Adenosine-5′-phosphate

Adenylic acid, or adenosine-5′-phosphate, is a nucleotide of particular biochemical interest. It is not only a component of ribonucleic acid, but also forms a part of the structure of many coenzymes and, as the di- and tri-phosphate, it is frequently involved in metabolic processes. Spencer[1] has reviewed the information at present available on the constituent components of the nucleotides from crystal structure analysis. In addition to the references cited by Spencer, one may also mention the brief note of Brown *et al.*[2] on a single projection of adenosine-3′-phosphate and the paper by Alver and Furberg[3] on the structure of cytidine-3′-phosphate. No nucleoside-5′-phosphate has been solved previously.

The unit cell parameters for adenylic acid are $a = 12{\cdot}77$ Å., $b = 11{\cdot}82$ Å., $c = 4{\cdot}88$ Å., $\beta = 92°\ 19'$, space group $P2_1$. Unidimensionally integrated data were collected on a Wiebenga-type camera using copper radiation.

The structure was solved by three-dimensional Patterson superposition methods based on identification of the phosphorus peak in the Harker section at $v = \frac{1}{2}$. It has been refined in projection on (010) and along [001] to R values of 13·7 per cent and 13·4 per cent respectively. Fig. 1*A* is an F_o synthesis showing $e(x,y)$ at the present state of refinement, and Fig. 1*B* is a perspective drawing of the molecule.

It may be noted here that the ribose ring is puckered in such a way that carbon 3′ is about 0·5 Å. out of the approximate plane formed by the four remaining atoms of the ring, lending further support to Spencer's hypothesis that the preferred conformations of the ribose ring are those with either atom 2′ or 3′ out of the plane.

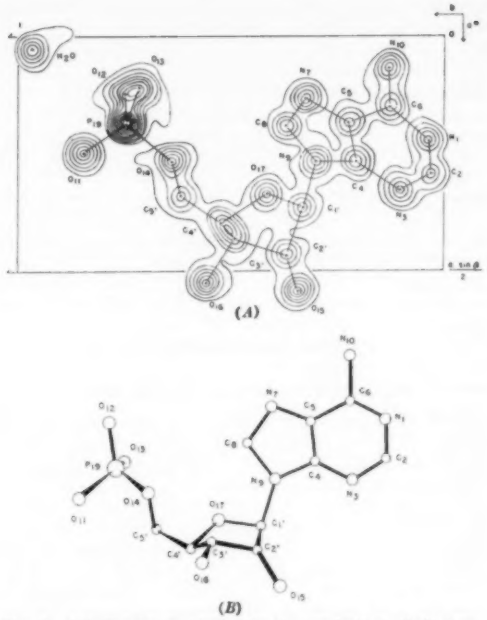

Fig. 1. (*A*) Fourier synthesis showing projection of electron density along [001]; contours at intervals of 2 e/Å.^{3}. (*B*) Perspective drawing of the adenylic acid molecule

The structure is being refined further with full three-dimensional data, and a complete account will appear elsewhere.

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Crystal Structure of Tetraphenyl*cyclo*butadiene Iron Tricarbonyl

There has been much recent interest[1–5] in the possible existence of the *cyclo*butadiene ring system in transition metal complexes, and a crystal structure determination[6] has verified that Criegee and Schröder's $C_8H_{12}NiCl_2$ is indeed tetramethyl *cyclo*butadiene nickel dichloride. The present investigation similarly verifies the conjecture[4,5,7] that the compound $Fe(CO)_3(C_6H_5C_2C_6H_5)_2$ of Hübel *et al.*[4] is tetraphenyl-*cyclo*butadiene iron tricarbonyl.

The yellow crystals, supplied by European Research Associates, are monoclinic with $a_o = 8{\cdot}93$ Å., $b_o = 18{\cdot}73$ Å., $c_o = 14{\cdot}10$ Å., $\beta = 92{\cdot}6°$, with four molecules per cell, and have extinctions corresponding to space group $P2_1/c$. Diffraction intensities were obtained by the stationary crystal–stationary counter technique[8] at rates as high as 500 a day using a General Electric *XRD-5* spectrometer equipped with single-crystal orienter and scintillation counter. A total of 2,306 reflexions, all the reflexions with $\lambda^{-1} \sin\theta \leqslant 0{\cdot}481$, were measured with a molybdenum $K\alpha$ source; of these, the strongest 1,100 have been used in the work so far.

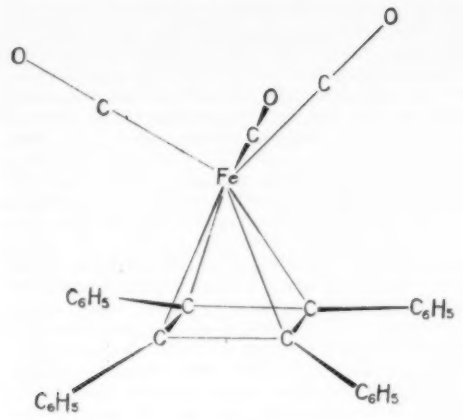

Fig. 1

The three-dimensional Patterson function clearly indicated the correct Fe—Fe vectors. Three-dimensional superposition based on the Fe position and recorded by means of the zero contour of the minimum function led to the correct structure except for two carbon atoms, one missing and one badly misplaced. An electron-density map then revealed the complete structure, and two further cycles of Fourier refinement have resulted in only relatively small further adjustments. The present agreement between observed and calculated structure factors is good, with discrepancy index (for the 1,100 reflexions used) of $R = 0{\cdot}145$. Further refinement is in progress.

The molecular structure is shown in Fig. 1. The *cyclo*butadiene ring is square planar to within the errors of the present determination of atomic positions, which, from the planarity and symmetry of the phenyl rings, linearity of the Fe—CO groups, and approximate agreement of the bond-lengths with standard values, are judged to be about 0·1 Å. Relative to an axis through the iron atom and the centroid, x, of the $(C_6H_5)_4C_4$ group, the $(CO)_3$ constellation has three-fold symmetry to within about 3° (with angles x-Fe—CO of about 120°) and the $(C_6H_5)_4$ constellation has fourfold symmetry to less than 5° except for twisting and bending of the phenyls out of the mean C_4 plane. The twists, all of the same hand in any one molecule, are about 30°, 30°, 30°, and 60°, and the bends, all away from the $Fe(CO)_3$, average about 10°. The phenyl of 60° twist is *trans* to a carbonyl. The intermolecular packing is generally good, but whereas no consistent intramolecular explanation is apparent for the asymmetry of phenyl twist, this irregularity does seem to correspond to certain irregularities in the packing.

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CHEMISTRY

Gaussian Wave Functions for the Hydrogen Molecule

Some time ago, Boys[1] showed that the troublesome potential energy integrals which arise in the quantum mechanical treatment of molecular problems are easily evaluated when the electronic wave function is expressed in terms of Gaussian functions:

$$\psi = \sum_k C_k \exp\left\{ - \sum_j a_j^k \mid r_j - \alpha_j \mid^2 \right\} \qquad (1)$$

(C_k, a_j^k are constants, α_j^k are constant vectors and r_j is the position vector of the j^{th} electron), and in terms of derivatives of such functions. The most complicated integrals can be reduced to expressions containing elementary functions and error functions (or derivatives of error functions).

Recently, Boys[2] showed that this is also true when correlation terms such as $\sum_j \sum_i g_{ji}^k \mid r_j - r_i \mid^2$ are added to the exponent of (1); while one of us[3] found independently that the potential energy integrals associated with Gaussian functions of the very general type:

$$\psi = \sum_k P_k \exp\ (-Q_k) \qquad (2)$$

(where Q_k is any positive definite quadratic form and P_k a polynomial in the electron co-ordinates) can be reduced to quadratures.

Gaussian functions of the form (1) have been used by Meckler[4] in calculations for the oxygen molecule. The more general functions which result from the addition of correlation terms are of interest because inter-electronic correlation is not easily taken into account when conventional wave functions (based on atomic orbitals) are used.

As a first step in the examination of wave functions of the form (2) we have carried out calculations for the ground-state of the hydrogen molecule. The wave functions were of the form:

$$\psi = \sum_{k=1}^{n} C_k [\varphi_k^+(1,2) + \varphi_k^+(2,1) + \varphi_k^-(1,2) + \varphi_k^-(2,1)]$$

$$\varphi_k^{\pm} = \exp\{-a_k(x_1^2 + y_1^2) - b_k(x_2^2 + y_2^2) - 2g_k(x_1x_2 + y_1y_2) - c_k(z_1 \pm \alpha_k)^2 - d_k(z_2 \pm \beta_k)^2 - 2h_k(z_1 \pm \alpha_k)(z_2 \pm \beta_k)\} \qquad (3)$$

which corresponds to electron orbitals of cylindrical symmetry with correlation and with 'floating' centres. The energy was minimized with respect to the parameters $C_k, a_k, b_k, c_k, d_k, \alpha_k, \beta_k, g_k, h_k$ at the equilibrium internuclear distance. Table 1 lists the binding energies obtained for $n = 3$, 4, 6 and 8.

Table 1

n =	3	4	6	8
No. of independent variable parameters	26	35	53	71
D (eV.)	4·30	4·42	4·52	4·58

The omission of the correlation parameters (g_k,h_k) led to poorer results (4·07 for $n = 3$ and 4·35 for $n = 8$).

The binding energies obtained with $n = 6$ and $n = 8$ are, so far as we know, higher than any other calculated values except those of James and Coolidge[5] (4·70); and it is worth noting that these authors used elliptical co-ordinates which are not suitable when the molecule contains more than two centres.

In spite of the large number of variable parameters the computing times (on a 'Mercury' electronic com-

puter) were relatively short: the evaluation of a typical matrix element, for example:

$$\int \varphi_{k^+}(1, 2)\, H \varphi_{k^-}(2, 1)\, d\tau$$

required less than 0·2 sec., the calculation of the energy for a given set of parameters varied from 13 sec. for $n = 3$ to 63 sec. for $n = 8$, and the corresponding total computing times were approximately 2 hr. and 14 hr. respectively.

A full account will be published elsewhere. Further work is in progress.

We wish to express our thanks to the director and staff of the London University Computer Unit for enabling us to use the facilities of the Unit.

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Non-Equilibrium Theory of Gas-Liquid Chromatography

In a gas-liquid chromatographic column the assumption of equilibrium demands that the rate constants for solute transfer between the gas and the liquid phases must be infinitely large and the flow-rates extremely small. In practical columns neither of these conditions is ever fulfilled; the kinetic rate constants are finite, and the use of extremely slow flow-rates is out of the question because of undesirably long retention times and pronounced longitudinal diffusion in the gas phase which enhances band broadening. This has led various workers to advance theories which take account of the effect of non-equilibrium conditions on band structure and column efficiencies[1–4]. Of particular importance is the van Deemter theory[4], which uses the driving force concept to describe the departure from equilibrium in terms of resistances to mass transfer in the gas and the liquid phases. The hypothesis of interfacial equilibrium involved in the theory is one the validity of which cannot be demonstrated directly by experiment. The existence of equilibrium at the interface would mean that if the concentration in the liquid phase at the interface is specified, the concentration in the gas phase at the interface is the same as if the two phases had been in contact for an indefinite length of time. Owing to continuous flow of carrier gas it is scarcely likely that the molecules once evaporated from the surface will get to the same spot again, and this, obviously, introduces conceptual difficulties regarding the nature of dynamic equilibrium at any point within the column. A natural consequence of the assumption of interfacial equilibrium is that once the diffusing molecules strike the interface, they pass into the other phase without experiencing any resistance at the interface itself. On the contrary, discrepancies between the calculated and observed rates of absorption suggest that there should be resistance to mass transfer at the interface[5–7]. From the point of view of the theory of absolute reaction-rates, there are sound theoretical grounds for postulating the existence of interfacial resistance. One can imagine an energy barrier which may involve the formation of an 'intermediate-activated state'; of all the molecules which strike the boundary surface only those which have the required energy of activation for diffusion surmount the energy barrier while the rest rebound into the gas phase, and furthermore the orientation of molecules may play a part.

A more general theory of the chromatographic process, which takes into account the whole gamut of diffusion processes and is not restricted to equilibrium conditions, has been worked out. The absorption and desorption processes are described by the simple laws of kinetic theory which can handle problems outside the domain of equilibrium in a fairly direct manner. The solution of the partial differential equations, when interpreted in terms of the first and second moments of the residence-time distribution-functions, leads to the following expressions for the retention time (t_R) and the height equivalent to a theoretical plate (H):

$$t_R = \frac{L}{u}\left(1 + \frac{a_2}{a_1}\frac{k_1}{k_2}\right) \qquad (1)$$

and:

$$H = \underbrace{2\lambda d_p + 2\gamma\frac{D_1}{u}}_{\text{Longitudinal diffusion}} + \underbrace{\frac{2}{3}\frac{\frac{a_2}{a_1}\cdot\frac{k_1}{k_2}\cdot d^2 f_1}{\left(1 + \frac{a_2}{a_1}\frac{k_1}{k_2}\right)^2}\cdot\frac{u}{D_1}}_{\text{Resistance to mass transfer}} + \underbrace{2\frac{\frac{a_2}{a_1}\left(\frac{k_1}{k_2}\right)^2}{\left(1 + \frac{a_2}{a_1}\frac{k_1}{k_2}\right)^2}\frac{a_2}{k_1\sigma}\cdot u}_{\text{Interfacial resistance to mass transfer}} + \underbrace{\frac{2}{3}\frac{\frac{a_2}{a_1}\frac{k_1}{k_2}\cdot d^2 f_2}{\left(1 + \frac{a_2}{a_1}\frac{k_1}{k_2}\right)^2}\cdot\frac{u}{D_2}}_{\text{Resistance to mass transfer}} + \underbrace{2\frac{a_2}{a_1}\frac{k_1}{k_2}\cdot\frac{D_2}{u}}_{\text{Longitudinal diffusion}} \qquad (2)$$

(GAS PHASE: Apparent diffusion, Molecular diffusion; LIQUID PHASE)

where a_2/a_1 is the ratio of the area of cross-section of the liquid to that of gas phase, d_{f_1} and d_{f_2} are the 'effective depths' of the gas and liquid phases, k_1 and k_2 are absorption and desorption-rate constants respectively, σ the surface area per unit column length, and the rest of the letters have the same meaning as described in van Deemter's theory.

The theoretical basis for the above expressions is too lengthy to be given here. Although diffusion in the liquid phase normal to the interface is of primary importance, the general treatment of the problem demanded the inclusion of diffusion parallel to the interface which is represented by the last term in equation 2. In gas-liquid chromatography, the resistance to mass transfer in the gas phase is usually assumed to be negligible as compared with that in the liquid phase. The resistance at the interface may be an important factor in the design of chromatographic columns particularly at high flow-rates and low specific surface areas. We are at present investigating the possibility of evaluating the expression for interfacial resistance.

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A Rapid Method for Qualitative Analysis of Volatile Mercaptan Mixtures

THE recently developed technique of flash exchange gas chromatography[1,2] has proved valuable for the determination of trace quantities of volatile aldehydes, ketones and acids. This approach has now been extended to the qualitative analysis of volatile mercaptans[3,4] by use of the exchange reaction between mercuric mercaptides and toluene-3,4-dithiol.

Mercuric mercaptides[5-7] (3–5 mgm.) are introduced into a glass capillary melting point tube and an equal weight of warmed (liquid) toluene-3,4-dithiol added. The tube contents are centrifuged to produce a slurry of mercaptides in toluene-3,4-dithiol. The open end of the tube is placed in the sample port of a gas chromatography unit and the lower part of the tube is heated at 245–260° for 15 sec. in an oil-bath. The exchange reaction takes place and C_1–C_4 saturated mercaptans flash into the unit for separation and identification.

In Table 1 are tabulated the retention times for mercaptans regenerated in the exchange reaction. The reaction is slow and only the highly volatile mercaptans escape the reaction mixture in sufficient amount to give good detector response (heating *n*-pentyl and *n*-hexyl mercaptides with toluene-3,4-dithiol produces no definite peaks).

Table 1. RETENTION TIMES FOR MERCAPTANS (*R*SH) REGENERATED FROM MERCURIC MERCAPTIDES WITH TOLUENE-3,4-DITHIOL

R	Retention time (min.) ¼ in. × 10 ft. Carbowax 1540, 80° *	¼ in. × 5 ft. Apiezon *M*, 60° †
H	no reaction	
CH_3	3·6	1·3
C_2H_5	4·8	2·3
iso-C_3H_7	5·1	3·3
n-C_3H_7	7·9	5·4
iso-C_4H_9	10·5	9·4
sec.-C_4H_9	8·6	8·4
tert.-C_4H_9	5·2	4·3
n-C_4H_9	13·9	12·9

* Flow-rate of helium, 35 ml./min. † Flow-rate of helium, 50 ml./min.

No evidence was found for the thermal decomposition of mercuric mercaptides to disulphides[7] under the conditions of the exchange reaction. Alkyl sulphide–mercuric chloride complexes react with toluene-3,4-dithiol to produce the sulphide along with small amounts of the corresponding mercaptans. Lead mercaptides give a much slower rate of reaction with toluene-3,4-dithiol than the mercuric mercaptides.

We wish to acknowledge the helpful interest of Prof. Arthur Furst (Stanford University) in providing several potential exchange reagents.

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Coloured Sulphur Deposits at Low Temperatures

F. O. RICE obtained green, purple and black deposits by chilling sulphur vapour at liquid-air temperature[1]. Several parameters of the experiments, predominantly the vapour temperature, determine the colour. With the help of equilibrium data of the vapour composition[2] Rice has concluded that the yellow deposits arise from cyclo-S_8, green ones from catena-S_8 and purple to black ones from S_2.

In the course of a series of investigations on unstable low-temperature deposits we have chosen the sulphur system as a model. A summary of our results follows[3].

Each apparatus consisted of four units in series: (1) sulphur reservoir with large liquid surface at temperature v_s° C.; (2) heated quartz-tube designed for multiple collisions of sulphur molecules with the wall at v_f° C.; (3) molecular beam defining orifice;

Table 1

ν_s ° C.	ν_f ° C.	ν_t ° C.	mgm. S/cm.² min.	Colour of deposit approximately 10μ thick Reflected light	Transmitted light
160	200	−188	0·3	yellowish-green	
160	275	−188	0·3	dark green	red brown
160	300	−188	0·3	blue green	red
160	380	−188	0·3	violet	yellow red
105	600	−188	0·01	green	reddish
145	600	−188	0·2	dark green	red
200	600	−188	1·6	violet	yellow red

(4) target (ice, sodium chloride, potassium bromide, quartz, aluminium, copper) in high vacuum at temperature ν_t° C. The experiments were performed under near equilibrium and widely varied conditions, for example, ν_s and sulphur vapour pressure, ν_f, ν_t, (liquid helium, hydrogen, air, ethylene, solid carbon dioxide, room temperature), residual gas-pressure, rate of sulphur deposition.

Coloured deposits (other than yellow ones) are formed at $\nu_f > 250°$ C., $\nu_t < -100°$ C., and at a target ambient pressure sufficiently low for essentially collision-free flow of molecules from orifice to target. The deposits have a rough surface and sandy texture below − 100° C.

Table 1 shows the correlation of colour with ν_f and rate of deposition.

Ultra-violet and visible spectra of green deposits are continuous with a maximum absorption at 507 mμ.

In the infra-red all the deposits lower the transmission considerably in the range 1–25μ, probably due to scattering. Coloured deposits exhibit a strong absorption-band at $14{\cdot}96\mu = 668{\cdot}5$ cm.$^{-1}$ (Fig. 1*a*) with a half-width of ~ 7 cm.$^{-1}$. This band grows linearly with deposit thickness (Fig. 1*b*), does not change in 150 hr. at − 190° C., but vanishes according to a first-order rate law with $t_{\frac{1}{2}} = 57{\cdot}7$ hr. at − 104° C. (Fig. 1*c*), and (extrapolated) $t_{\frac{1}{2}} = 18$ min. at −78° C. Deposits which have been above − 78° C. do not redevelop infra-red absorption or colour on re-freezing to − 190° C.

This infra-red band is without any doubt due to the unstable sulphur form (method of purification and other arguments[3]). There is no measurable qualitative correlation of infra-red spectrum with colour. In matrix experiments with xenon (Xe : S = 500) only green deposits are formed with the same band at 668 cm.$^{-1}$.

At a micro-wave frequency of 9,500 Mc./sec. no paramagnetic resonance is observed with 3·2 mgm. of green sulphur deposit at − 188° C. in the cavity between 2,000–7,000 gauss (line width 100 gauss). An upper limit of 0·01 per cent detectable 'free spins' relative to the deposited number of S-atoms follows from this observation (measurements by Mr. F. Waldner, Physics Institute of the University of Zurich).

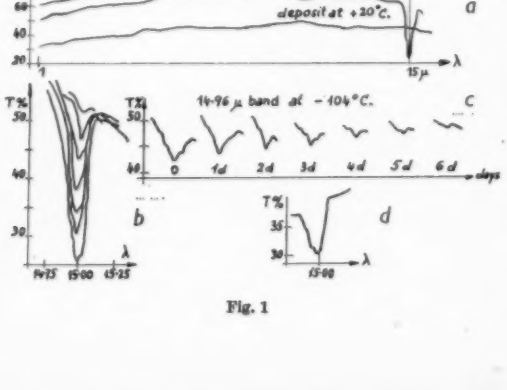

Fig. 1

Deposits of different colour have an electrical resistivity of 2×10^6 Ω cm. at − 188° C. compared with 10^{18} Ω cm. for normal sulphur. No photosensitivity with near ultra-violet and visible light is observed.

At room temperature deposits on sodium chloride formed below − 100° C. can be removed as coherent, shiny films which collapse elastically to a fortieth of their surface-area. They are 30 per cent soluble in carbon disulphide 2 hr. after warming up.

The experiments with a xenon matrix demonstrate the existence of trapped S_2, since this is the only sulphur constituent of the molecular beam. The infra-red absorption at 668·5 cm.$^{-1}$ must, therefore, be attributed to an induced vibrational transition of S_2 in the asymmetrical quasi-solid environment which has not been communicated before. There is a shift of 53 cm.$^{-1}$ from the fundamental $\omega_0 = 721{\cdot}6$ cm.$^{-1}$ in the gaseous state[4]. This fact and a barely visible fine-structure (Fig. 1*d*) are also characteristics of induced absorptions in liquid or solid oxygen[5]. S_2 is also responsible for the green colour of the xenon matrix. With the deposits of pure sulphur alone additional colours and a not very much stronger infra-red band are observed than in the very dilute S_2–Xe matrix. This can be understood by the following argument. S_2 molecules arrive at the target with enough kinetic energy to align themselves, combine, and form unoriented chains of variable length before attaining the target temperature. All these S_n-chains ($n = 3, 4 \ldots 8 \ldots$) together essentially show continuous infra-red absorption in the range studied. They form a 'self-matrix' which traps S_2 molecules at a concentration which is dependent on the parameters of deposit-formation, but which is constant under specified conditions.

Whereas this mechanism accounts for the transformation of most of the S_2 molecules on the target, the explanation of the different colours must start from the observation that no direct relation of the molecular composition of the deposits with colour exists. Infra-red absorption attributable to S_3 or S_4 . . . or S_8 . . . is not detected since no band, except at $14{\cdot}96\mu$, shows up clearly. Therefore the colour must be due to the physical pattern of aggregation of the sulphur on the target, that is, to selective scattering at polymer-clusters of roughly equal diameter[6]. Their size (~ 1000 Å.) depends on the rate of deposition or vapour temperature (Table 1). Reflected and transmitted light have complementary colours in agreement with this interpretation. Above − 100° C. the clusters combine and merge into a film covering the target evenly. The rough surface changes to a shiny one. Trapped S_2 molecules are set free to add to longer chains. On freezing again no S_2 absorption or colour-changes are detectable. The result of the paramagnetic resonance measurements is consistent with these interpretations[7]. The biradical character of the chains is probably responsible for a much lower electrical resistivity, the electrons in antibonding π-orbitals being largely delocalized along the chains.

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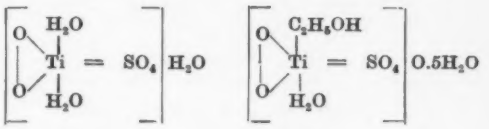
Nature of the Colour-forming Species in Peroxy Titanium Sulphate

Although titanium is determined colorimetrically in aqueous sulphuric acid medium in presence of excess of hydrogen peroxide, the nature of the colour-forming species is not known definitely. Schwarz[1] suggested that the colour was due to the peroxo–disulphato titanate anion $[O_2Ti(SO_4)_2]^{2-}$. On the other hand, Jahr[2] and later Gastinger[3] considered that the colour of the compound was due to the peroxy titanyl cation $[TiO_2\ aq.]^{2+}$, and suggested the following equilibrium in solution:

$$\underset{\text{Coloured}}{[TiO_2\ aq.]^{2+}} + H_2O = \underset{\text{Colourless}}{[TiO\ aq.]^{2+}} + H_2O_2 \qquad (1)$$

Schaeppi and Treadwell[4] attributed the colour to O_2TiSO_4 or $[O_2Ti(SO_4)_2]^{2-}$, whereas Babko and Volkova[5] represented the coloured complex ion as $[Ti(H_2O_2)]^{4+}$. Mori, Shibata, Kyuno and Ito[6] regarded the coloured species as $[TiO_2\ aq.]^{2+}$ or $[Ti(OH)_2(H_2O)(H_2O_2)]^{2+}$, assuming the co-ordination number of titanium to be four. Thus, a variety of constitutions has been proposed to explain the colour-forming species of the titanium complex, based on the investigations carried out in dilute sulphuric acid medium, but the complex has not been isolated so far.

We prepared a concentrated solution of peroxy titanium sulphate by the reaction of a freshly prepared titanium hydroxide with a cooled mixture of the calculated quantity of sulphuric acid and excess of hydrogen peroxide. The solution, when evaporated at 25° C. by passing dry air through it, gave a red-coloured solid peroxy complex of the composition $TiO_2SO_4.3H_2O$. The best sample prepared so far was 95 per cent pure, the balance being $TiOSO_4.3H_2O$. The complex contained a true peroxy group as shown by Riesenfeld Liebhasky test. It is amorphous and highly soluble in water and in absolute alcohol, but hydrolyses on dilution with water. The hydrolysis of the compound in aqueous solutions is prevented by dilute sulphuric acid. From various physico-chemical studies the solid compound is assigned the structure (I):



(I) (II)

The compound in dilute sulphuric acid has an absorption maximum at 410 mμ, as also observed by Reeves and Jonassen[7], while in absolute alcohol it is at about 390 mμ. The compound when dissolved in absolute alcohol is found to have the structure (II). The molecular weight as determined by the cryoscopic method in 1 *N* sulphuric acid gives a value of 178, instead of the theoretically calculated value of 230. This shows a dissociation of the complex in solution to the extent of 28 per cent at about − 2° C. To find out the exact nature of the dissociation of the complex, electromigration, conductivity and spectrophotometric investigations were carried out on the complex. The compound in 2 *N* sulphuric acid medium showed very little electromigration towards the cathode, which is in agreement with the finding of Schaeppi and Treadwell[4]. This indicates that the complex has practically zero charge. This observation was confirmed by a conductivity experiment, from which it was found that the conductivity of a 0·5 *M* solution of peroxy titanium sulphate was practically the same as that of the solvent. Thus the coloured complex is probably dissociated into two neutral molecules:

$$TiO_2SO_4 + H_2O = TiOSO_4 + H_2O_2 \qquad (2)$$

This type of dissociation was confirmed spectrophotometrically by the molar ratio method. It was found that the dissociation of the complex ($0{\cdot}325 \times 10^{-3}$ *M*) in 1·6 *N* sulphuric acid, in accordance with equation (2), was 40 per cent, whereas in 6·8 *N* sulphuric acid it was 33 per cent, both at 25° C. All studies show that the coloured compound in dilute sulphuric acid medium dissociates mainly according to equation (2).

The present work does not support the observation of Reeves and Jonassen[7] that the sulphate ligand takes no part in complex formation. Further, there is no evidence so far of the formation of the complex ions suggested by Schwarz[1] and by Babko and Volkova[5].

The coloured complex, represented by the structure (I), satisfies the normal co-ordination number of six for titanium. This is also supported by the fact that the two co-ordinated molecules of water are difficult to remove, when the solid complex is subjected to evacuation for a long time, whereas the third water molecule is more easily removed. The complex gives off ozone and oxygen on keeping, indicating the presence of the strained triangular ring of the peroxy group of the complex. The sulphate group occupying two co-ordination positions[8] in the complex is likely to facilitate its hydrolysis in dilute aqueous solutions, forming mainly hydrated titanium peroxide and sulphuric acid.

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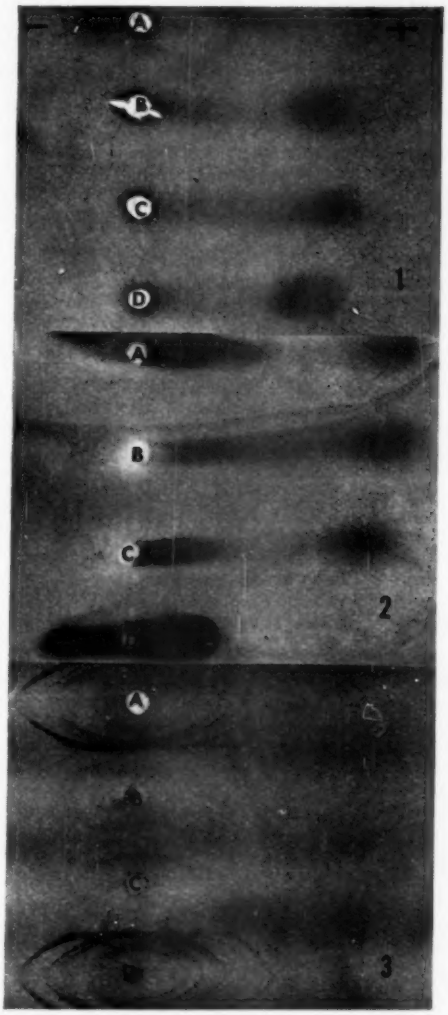
BIOCHEMISTRY

Mucopolysaccharides of Serum and Pleural Fluid

The use of paper electrophoresis has enabled several investigators to establish the presence of acid mucopolysaccharide components in various tissue extracts[1,2] and urine[3]. Two such substances, staining with toluidine blue, were found in human aortic tissue[4]; concentrated human urine contained three components which stained with alcian blue[5]. The latter all appeared to migrate more rapidly than the albumin fraction, with the fastest component having the same mobility as chondroitin sulphate *A*. In the present work, which is primarily concerned with the electrophoretic characteristics of various body fluids in agar, a component was noted in the perchloric–phosphotungstic acid precipitate of both serum and pleural fluid which possessed staining and electrophoretic properties similar to those of acid mucopolysaccharide in urine.

The method of Winzler *et al.*[6] was used for the precipitation of mucoproteins from fresh normal human serum, normal urine, and from pleural fluid obtained from a patient ill with cholecystitis. To 2·0 ml. of each specimen was added 8·0 ml. 0·75 *M* perchloric acid. In 10 min. the precipitate which appeared in the serum and pleural fluid was removed by filtration; the urine sample remained clear. 2 ml. of 5 per cent phosphotungstic acid in 2 *N* hydrochloric acid were added to the filtrates and acidified urine, the tubes were centrifuged, the sediments washed twice with the phosphotungstic acid and resuspended in 2·0 ml. veronal buffer, *p*H 8·6, 0·03 ionic strength. Electrophoretic separation was carried out at refrigerator temperature with techniques similar to those described by Zak and Jarkowski[7] and by Crowle[8]. Slides (2 × 3 in.) were coated with 2 per cent 'Ionagar' (Oxo, Ltd., London) and dried at 37° C. Plastic tape was cut into strips approximately 3 mm. wide and layered on the long edges of the slides. Filter paper wicks were placed at each end and 4·0 ml. melted 1 per cent agar in the veronal buffer was poured between the taped edges. A 'Plexiglas' matrix with four holes was lowered until it rested on the tape strips. Following a period of 20 min. in a refrigerator, the agar was removed from the holes by vacuum suction. One 0·01 ml. of each sample was placed in separate holes by capillary pipette. A solution of orange *G* was added to a sixth hole located near an edge of the matrix and close to the cathode-end of the slide. Current (9 V./cm.) was applied until the orange *G* moved 2·0 cm., which required approximately 20 min. The matrix was removed and the slides fixed and stained for mucopolysaccharides[5], lipoprotein or mucoprotein[9] components.



Figs. 1–3. Electrophoretic properties in agar of serum, pleural fluid and urinary acid mucopolysaccharide. Fig. 1 was stained with alcian blue and azocarmin; Fig. 2 with α-naphthol-phenylenediamine; Fig. 3, immunoelectrophoresis with rabbit anti-human serum placed in troughs adjacent and parallel to the separated fractions. Untreated serum: 1,*A*, 3,*D*; acid precipitated serum: 1,*B*, 2,*A*, 3,*C*; acid precipitated urine: 1,*C*, 2,*B* untreated pleural fluid: 2,*D*, 3,*A*; acid precipitated pleural fluid: 1,*D*, 2,*C*, 3,*B*

The characteristics of mucopolysaccharide present in perchloric–phosphotungstic acid precipitated serum and pleural fluid were quite similar to those of acid-treated urine as shown in Fig. 1. The substance appeared to migrate 2–3 times faster than the albumin fraction and stained with both alcian blue and α-naphthol-phenylenediamine reagent. The specificity of the former dye for acid mucopoly-

saccharide was shown by Heremans *et al.*[5] and the present experiments confirm their observation. The alcian blue stained only the fast-migrating material (Fig. 1) whereas the major substance of the treated serum and pleural fluid appeared to be mucoprotein (Fig. 2), which resembled the immuno-electrophoretic fraction *X* of de Vaux St-Cyr[10]. This was confirmed by extending the period of electrophoresis until the indicator dye had migrated the full length of the slide. The mucoprotein fraction then appeared to have a mobility similar to α_1-globulin of untreated serum. Proteins and lipoproteins were apparently removed by the isolation procedures; only the untreated serum (Fig. 1*A*) and pleural fluid (not shown) counterstained with azocarmin and with oil red *O*. Immuno-electrophoresis (Fig. 3) suggested that the mucopolysaccharides were not antigenic in the rabbit since precipitation did not occur in the corresponding area of migration. However, this apparent lack of antigenicity may be due to alteration of the material during acid precipitation or to a difference in complexity of the substance as it existed in the serum which was used to immunize the animal. Indeed, the fast-migrating mucopolysaccharide was not detectable in untreated serum (1, *A*) or pleural fluid. Within the necessary limitation placed on the extent of separation, all fractions of the latter two specimens appeared to contain mucoprotein (2, *D*). Following acid precipitation, the α_1-globulin was the only main serum fraction staining with the dye. This suggested that the mucopolysaccharide represented prosthetic groupings split from the mucoproteins of other serum fractions during acid treatment. Although the present evidence indicated this possibility, it may be emphasized that concentration of the samples was not attempted and, therefore, the existence of 'free' mucopolysaccharide cannot be excluded since the concentration may be below the limits of detectability. Regardless of the origin, it would appear important to gain further information concerning the biological properties of the isolated mucopolysaccharide.

The use of agar in electrophoretic studies such as these offers certain advantages over paper methods. Separation of the various components is accomplished more rapidly without loss of detail, and the apparatus is of very simple design. The elimination of elution procedures prior to immunological investigation also favours the present method.

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A New Technique for studying the Quantitative Aspects of Cytochemical Reactions and its Application to the Feulgen Reaction

Quantitative microspectrophotometry of cytochemically stained substances in cellular structures should be based on a knowledge of the stoichiometry of the reactions involved and the conditions influencing it. Thus far independent chemical analysis of individual cellular structures to study the quantitative relation between colour and amount of stained substance in general is not possible. This relation can be studied on a macro-scale by incorporating the substance in a kind of artificial section or model system which makes it possible to carry out the staining procedure under cytochemical conditions. For this purpose thin cellulose films were chosen. The extinction of the film after the staining procedure is proportional to the mass of the stained substance per unit area in the film. This mass is proportional to the amount of cellulose in the same area, provided the substance is uniformly distributed.

Since the cellulose films can be made of reasonable strength this amount of cellulose per unit area can be found by punching out pieces of known diameter in the measured region and weighing the dried punches. If no loss of substance occurs during preparation and staining of the model system the mass of stained substance per punch weight can be derived from the calculated composition of the film as a whole.

If an analysis method for the substance is known or can be developed this method can be used to analyse individual punches directly at the end of the staining procedure. Such analysis makes the method independent of initial irregular distribution or irregular loss of the substance in different areas of the film. Another advantage of the use of cellulose films is their favourable optical properties, which permit the study of spectra of the incorporated substance down to 230–240 mμ.

To demonstrate the value of this system, deoxyribonucleic acids were incorporated in a cellulose film.

The model system was prepared from a solution of deoxyribonucleic acid in viscose spread in a thin layer on a glass plate. This layer was dried somewhat at 60° C. and converted in a cellulose film in which deoxyribonucleic acid was quantitatively incorporated through the action of a mixture of 3 vol. methanol and 1 vol. formic acid. The films were fixed overnight in a mixture of 30 per cent formaldehyde and methanol (1/9 v/v). After having been kept in methanol for some days, the films were subjected to the Feulgen reaction (hydrolysis for 12 min. at 60° C. in 1 *N* hydrochloric acid). The stained films as well as their appropriate controls were cut into small strips. These strips were transferred to a spectrophotometer cell filled with *o*-xylene and were held mechanically in a stretched position. After the measurement of the extinction a round piece of each strip was punched out in the region that had been traversed by the light beam. The dried punches were weighed and analysed for inorganic phosphorus after digestion in a perchloric acid–hydrogen peroxide mixture. The standard deviation of the mean of the values of phosphorus content per mgm. dry cellulose of the unstained punches could be kept within 1 per cent.

The result of an experiment in which films of various concentrations of deoxyribonucleic acid were used is given in Fig. 1. From each film strips were cut,

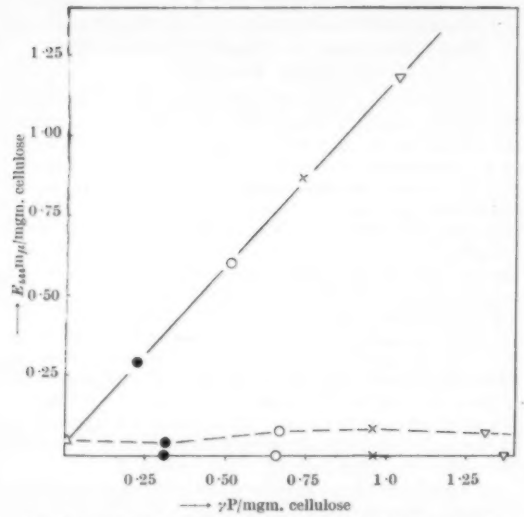

Fig. 1. Schiff-stained deoxyribonucleic acid–cellulose membranes. ———, Hydrolysis; – – – –, no hydrolysis. The phosphorus content of the unhydrolysed and unstained membranes ($E < 0{\cdot}005$) are plotted on the abscissa. The dry weight of the punches (diam. 5·49 mm.) varied between 0·55 and 0·80 mgm.; their thickness in xylene was found to be in the range 60–80μ

which were stained omitting the hydrolysis procedure. The results show that a linear relation exists between the deoxyribonucleic acid content after staining and the value of the extinctions measured. This linearity is not influenced by the formaldehyde treatment and in general is independent of the way in which the films are prepared from the viscose layer. Since the points in the graphs are based on mean values from five to six punches, an impression of the accuracy of the method can be gained from the standard deviations for the quotient $E/\gamma P$ of the individual punches, given in Table 1. These results give further proof of the validity of the Feulgen reaction as a quantitative method for deoxyribonucleic acid.

Table 1

Groups of punches*	γP/mgm. dry cellulose (calculated)	Mean $E_{444}/\gamma P$
●	0·321	1·12 ± 0·035† (6)‡
○	0·70	1·10 ± 0·033 (6)
×	1·00	1·13 ± 0·033 (5)
▽	1·40	1·10 ± 0·02 (6)

* See Fig. 1. † Standard deviations. ‡ Number of punches.

Cytophotometry of Feulgen-stained cell nuclei in general is carried out on an empirical basis, working with arbitrary units of deoxyribonucleic acid content within a certain experiment[1]. Based on the hypothesis of the constancy of the amount of deoxyribonucleic acid in cell nuclei within one species, macro-analysis of suspensions of cell nuclei in this particular case could be used to some extent to investigate the stoichiometric relations involved, as was done by Ris and Mirsky[2]. The use of an artificial section may facilitate the study of the factors influencing the stoichiometry of the Feulgen reaction. Such studies may contribute to the solution of the problem if the deviations from the constancy hypothesis, still found by microspectrophotometry of Feulgen-stained nuclei, are based on real variations in deoxyribonucleic acid content.

The system seems suitable for testing the behaviour of other aldehyde reagents instead of the Schiff reagent in the Feulgen reaction. In this respect we investigated the behaviour of the blue thionine–SO_2 aldehyde reagent reported previously[3]. These studies show a similar linear relation between extinction (at 590 mμ) and deoxyribonucleic acid amount as was found for the Schiff reagent.

Investigations with the aid of the model system may facilitate the finding of staining conditions that reflect to some extent the positions of the aldehyde groups in the apurinic acids from several types of deoxyribonucleic acid.

Since its value has now been proved for the Feulgen reaction it appears reasonable to extend the method to compounds other than deoxyribonucleic acid for which a study of the stoichiometric relation cannot be based on a constancy hypothesis.

In its present form the method is limited to the investigation of quantitative cytochemical behaviour of alkali-stable substances. Removal of the excess alkali from the viscose just before mixing and film formation seems possible. In addition other cellulose derivatives with film-forming potentialities are known to exist so that the present limitations appear to be surmountable.

Part of this work has been published as a thesis for a Ph.D. degree by one of us[4].

Full details will be published elsewhere. We wish to thank Miss J. Allaart for technical assistance.

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Biological Significance of Peptide containing Hydroxyproline during the Development of Carrageenin Granuloma

Although it is generally accepted that free hydroxyproline cannot be utilized for the synthesis of collagen[1–3], we have demonstrated in previous papers a close interrelation between the level of free hydroxyproline in tissues of rats[4], or in the body of chicken embryos[5], and the rate of collagen synthesis (after cortisone administration). In a recent paper Green and Lowther[6] presented proofs that the formation of protein-bound hydroxyproline proceeds by way of 'activated' proline which is hydroxylated during the incubation in granuloma slices to form 'activated' hydroxyproline. The possibility of such a hydroxylation of proline *in vitro* has been demonstrated recently[7]. Some hypotheses dealing with collagen synthesis presume in connective tissue the presence of peptide with hydroxyproline, the biochemical existence of which has not yet been proved. In an ultrafiltrate of extracts from bovine embryonic tissues homogenates (subcutis, growth-zone of bones) we found repeatedly several peptides containing hydroxyproline. Their chromatographic and other biochemical characteristics will be published elsewhere (Kobrle, V., and Chvapil, M.).

In this study are presented further findings concerning the biological significance of peptide or peptides containing hydroxyproline.

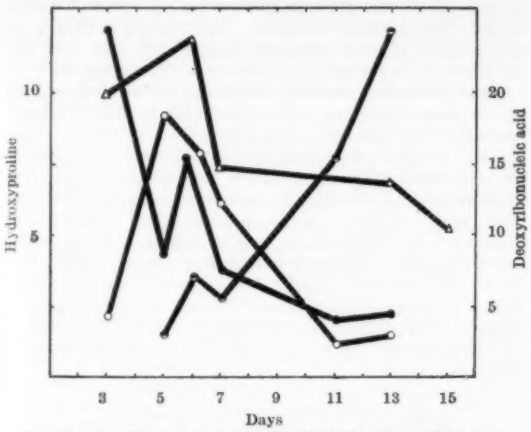

Fig. 1. Changes in free and peptide hydroxyproline, total tissue hydroxyproline and deoxyribonucleic acid concentration during the development of carrageenin granuloma. ○, Peptide hydroxyproline; ●, free hydroxyproline; ◒, total tissue hydroxyproline; △, deoxyribonucleic acid. Results are presented in μgm./100 mgm. of dry substance

For this reason carrageenin granuloma in guinea pigs was produced by the usual technique[8] under simultaneous penicillin and streptomycin treatment. On individual days of the development of the granuloma until the fifteenth day, the tissue was collected, homogenized with the aid of air as fluid and with a Potter and Elvehjem apparatus, extracted 60 min. with 0·14 *M* sodium chloride. The whole homogenate was centrifuged at 15,000*g*; the proteins in the supernatant were separated by means of ultrafiltration in metal pressure ultrafilter using a membrane supplied by Nojax Cellulose Casinas, Visking Corp., Chicago. The absence of proteins in ultrafiltrate was always re-examined. An aliquot of the ultrafiltrate was hydrolysed (6*N* hydrochloric acid, 140° C., 3 hr.). In hydrolysed and non-hydrolysed parts of the filtrate we determined colorimetrically the amount of hydroxyproline[9], and the total amount of iminogroups[10] polarographically. In an aliquot of the whole homogenate we determined after acid hydrolysis the total hydroxyproline[9], and in another aliquot the amount of deoxyribonucleic acid[11]. Two independent series of experiments were carried out with similar results.

Results of a second series of experiments are summarized in Table 1 and Fig. 1.

It is evident that in the first days of granuloma development there occurs an accumulation in tissue of peptide containing hydroxyproline which forms 20–65 per cent of total filtrable hydroxyproline. It is worth mentioning that the changes in peptide–hydroxyproline follow the changes in amount of deoxyribonucleic acid in tissues. In good agreement with the findings of Jackson[12], the maximum of collagen accumulation is 7–10 days.

Table 1

	Duration of experiment (days)					
	3	5	6	7	11	13
Free hydroxyproline*	12·4	4·4	8·04	3·84	1·94	2·27
Peptide hydroxyproline	2·2	9·3	8·05	6·18	1·16	1·53
Percentage of peptide-hydroxyproline†	15	68	50	60	37	40
Total tissue hydroxyproline		162	374	289	780	1,235
Deoxyribonucleic acid	2,020	1,550	2,380	1,720	973	1,480

* Results are presented in μgm./100 mgm. of dry substance.
† Percentage of peptide-hydroxyproline is calculated from total ultrafiltrable hydroxyproline.

The level of free hydroxyproline in granuloma tissue diminishes at first, and then alters simultaneously with peptide.

The presence and the amount of peptide with hydroxyproline in an ultrafiltrate does not depend on whether the tissue homogenate was heat-treated (100°, 10 min.) or not. For this reason we exclude the possibility that peptide hydroxyproline is the source of collagen degradation by some tissue enzymes.

We suppose, therefore, that the accumulation of peptides containing hydroxyproline in their molecule in carrageenin granuloma is connected with early collagen protein synthesis, or stimulated collagen production bound on the presence of fibrillogenic connective tissue cells. The interrelation of free hydroxyproline and peptide–hydroxyproline is not yet quite clear. The elucidation of these questions is the task of further studies.

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Flavonoid Compounds in the Inflorescence of *Helichrysum arenarium*

In a previous paper[1] we reported the isolation and identification of three crystalline compounds from the inflorescence of *Helichrysum arenarium*. We isolated from the alcoholic extract the salipurposide, naringenin-5-glucoside (5,7,4′-trihydroxyflavanone-5-glucoside), $C_{21}H_{22}O_{10}\cdot 2H_2O$, m.p. 219–224°, yield 0·25 per cent, acetyl derivative m.p. 174–177°. Until then, this flavanone glucoside had been found only in *Salix purpurea* and *Acacia floribunda*.

We also isolated the apigenin (5,7,4′-trihydroxyflavone), m.p. 346°, yield 0·03 per cent (triacetyl derivative m.p. 179·5–181·5°, dimethyl derivative m.p. 155–156°), which occurs in dried flowers in a free state. After hydrolysis with dilute sulphuric acid of a syrupy fraction, which could not be obtained in crystalline state, we isolated the kæmpferol (3,5,7,4′-tetrahydroxyflavone) $C_{15}H_{10}O_6$, m.p. 276–278°, yield 0·1 per cent; triacetyl-derivative m.p. 177°.

Now we have obtained evidence that kæmpferol occurs in *Helichrysum arenarium* as a glucoside.

Using paper partition chromatography we have shown, besides salipurposide, the presence of still two other flavonoid compounds. (Whatman No. 3 circular-paper chromatography, ethyl acetate–formic acid–water, 10 : 2 : 3, $R_F = 0{\cdot}84$, $R_F = 0{\cdot}77$.) One compound could be isolated in pure state, $C_{21}H_{20}O_{11}$,

m.p. 171–175°, $R_F = 0{\cdot}84$, yield 0·1 per cent (calc. C, 56·25; H, 4·50; found: C, 55·89; H, 4·54) and identified with kæmpferol-3-glucoside (astragaline). This substance hydrolysed with acid gives a compound with m.p. 278–280°, $R_F = 0{\cdot}87$ (Whatman No. 1, *n*-butanol–acetic acid–water, 4 : 1 : 5) identified with kæmpferol, and glucose identified by paper chromatography ($R_F = 0{\cdot}18$) and its osazone.

By methylation and thereafter hydrolysis a crystalline compound, m.p. 148–149°, identical with 5,7,4′-trimethylkæmpferol was isolated. This provides evidence of location of the glucosidic linkage at position 3.

Our R_F value distinctly differs from the observed one by Hörhammer[2], $R_F = 0{\cdot}67$, in the same solvent system.

The simultaneous occurrence in the same plant of three grades of oxidation of the flavonoid nucleus—flavanone, flavone, flavonol (naringenin, apigenin, kæmpferol)—is interesting. One may suggest the possibility of biosynthesis of apigenin from naringenin and of kæmpferol from naringenin or apigenin.

Analogous oxidations were realized by iodine, selenium dioxide and hydrogen peroxide *in vitro*.

The results of pharmacological study now under investigation will show if the flavonoid compounds which we have isolated are responsible for the cholagogue activity observed for the drug.

The crude extract, containing apigenin as major compound, showed in preliminary trials cholagogue activity, and another, containing salipurposide and kæmpferol-3-glucoside, showed a spasmolytic activity and a feeble cholagogue one (A. Szadowska, Department of Pharmacology, Med. Academy; more extensive results will be published elsewhere).

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PHYSIOLOGY

Improvement of the Intravenous Glucose Tolerance Test

Glucose is carried to its metabolic sites directly by plasma water and only indirectly by red blood cells. Accordingly, the intravenous glucose tolerance test would convey greater information if it were reported in terms of plasma glucose concentration. Yet whole blood is currently used in such determinations. Whole blood contains glucose in both red blood cells and plasma, and since the transport between them is moderately slow the concentrations are generally different when blood glucose-levels are rapidly changing. For example, if plasma water glucose concentration is suddenly raised from 100 to 200 mgm. per cent, data of Bowyer and Widdas[1] indicate that it would take 17 min. for the glucose concentration in the red blood cell water to reach 190 mgm. per cent.

Slow transport brings into question interpretations based upon a simple exponential model for the intravenous glucose tolerance test[2]. For if the glucose concentration curve for the whole blood is a simple exponential function, and if slow transport occurs, then the plasma glucose curve cannot be a simple exponential function. The complex shape of the plasma glucose concentration curve can best be understood in terms of a model shortly to appear[2].

This complex model depends upon inferences about plasma glucose concentrations, because experimental data are not available. The utility of the model would be materially enhanced by direct experimental determinations of plasma glucose concentrations during the intravenous glucose tolerance test. Such determinations could readily be obtained by immediate refrigeration and prompt centrifugation of blood samples to retard transfer. Such refrigeration could be obtained by drawing the blood through polyethylene tubing immersed in ice water. The difficulties of intra-arterial cannulation can be obviated by using venous blood after warming the arm.

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Abrupt and Delayed Effects of Insulin on the Distribution Space of Glucose in the Dog

It has been proposed that insulin increases the permeability of cell membranes of animals to glucose[1]. If this is so, an increase induced by insulin in the volume through which glucose can disperse could occur as a result of the addition of intracellular spaces to those through which glucose is distributed without the added insulin. The method of successive tracer injections[2] provides a means for determining, at each injection time, the amount of glucose which intermixes rapidly with the plasma glucose. From this value the corresponding apparent volume or space of distribution can be calculated, assuming that the concentration of plasma glucose is uniform throughout the volume. In this communication these procedures were used to determine the immediate and later effects of injection of insulin and of infusion of insulin on the glucose distribution space of the dog.

Five experiments were performed on four normal dogs anæsthetized with nembutal. Glucose, uniformly labelled with carbon-14, was injected intravenously through an indwelling polyethylene cannula inserted 3–4 in. into the tibial vein. Samples were withdrawn from the inferior vena cava, which was catheterized via the femoral or saphenous vein. Insulin was given

Table 1. Effect of Insulin on Level of Plasma Glucose (*C*, mgm./100 ml.) and Volume of Glucose (*V*, per cent of body-weight) in Normal Anæsthetized Dogs

No. of experiment	Weight of dog (kgm.)	Mode of administration of insulin	Before insulin				Times of injection of ^{14}C-glucose after injection or start of infusion of insulin					
			100 min.		40 min.		23–25 min.		60–69 min.		108–114 min.	
			V	*C*	*V*	*C*	*V*	*C*	*V*	*C*	*V*	*C*
1	12·0	Injection, 1 u./kgm.	17·3	115·7	15·7	106·9	*		46·8	30·0		
7*A*	23·6	Injection, 0·29 u./kgm.	18·0	84·2	14·7	84·2	44·1	26·2	33·3	43·5	24·1	85·4
3	11·5	Infusion, 0·008 u./kgm./min.	23·4	89·1			24·3	63·2	44·4	31·4	30·3	38·7
6	16·9	Infusion, 0·0038 u./kgm./min.	19·8	106·2	22·3	97·4	17·7	55·1	182·3†	33·2	29·0	32·5
7	25·0	Infusion, 0·0018 u./kgm./min.	21·8	77·8	21·6	77·8	20·9	64·0	31·0	55·5	32·0	28·2

* In this experiment volume of glucose was measured at injection time. It was 12·5 per cent of body-weight.
† This determination is based on a specific activity versus time curve of a very poor fit. Therefore it may represent a gross over-estimation of volume of glucose. It is included in order to present unselected results.

either as a single intravenous injection, or as a constant intravenous infusion from a calibrated burette. Glucose was determined by the Somogyi–Nelson method[3]. Specific activity of plasma glucose was measured by preparing glucosazones, the radioactivity of which was measured in a windowless flow-counter. In experiments 1 and 3 (Table 1) Insulin-Toronto was used, whereas in the others crystalline Insulin-Lilly was injected.

Results are shown in Table 1. It can be seen that the injection of insulin resulted in an increase in the apparent glucose volume in all experiments. The effect is a delayed one and is not strictly related to the changes of level of blood glucose. It can be seen in experiments 3 and 6 that, although the level of blood glucose had started to drop, the increase in apparent glucose volume was delayed. In experiment 7*A*, the level of blood glucose had returned to normal, while the apparent glucose volume was still somewhat above the pre-insulin level. In experiment 1 the glucose volume was determined at the time of insulin injection and was found to be slightly less than the pre-insulin volumes. This is in accord with our earlier findings[2].

The present experiments do not indicate whether the effect of insulin on the apparent glucose volume is a specific one, or whether it results from the hypoglycæmia evoked by insulin. Experiments to elucidate this point are in progress.

In support of the present findings it has been shown that insulin increases the volumes of distribution of galactose and of some pentoses in the eviscerated dog[4], in isolated tissues[5], and in perfused organs[6]. Under certain conditions an accumulation of free glucose in the isolated rat diaphragm was also noted when insulin was added to the incubation medium[7].

The effect of insulin on the apparent glucose space was studied by Drury and Wick[8], who compared two groups of eviscerated-nephrectomized rabbits. One group served as controls, while the other received 10 units kgm./hr. of insulin. No difference in the apparent glucose spaces was found. Each of our experiments with successive tracer-glucose injections was performed on a single dog, so that inter-animal variation was avoided. All our results show an increase in the apparent glucose space during insulin hypoglycæmia, but not immediately after the start of the injection of insulin. The enlarged space appears to exceed that of the extracellular body-water.

This finding modifies the conclusions from earlier experiments, in which attempts were made to determine the effects of insulin on the rates of appearance and disappearance of glucose from the plasma and associated tissue fluids (central compartment). All these methods except one[3] involved the assumption that the glucose space remained constant. The observed increase in the apparent glucose space following injection of insulin leads to an under-estimation of the rate of appearance or an over-estimation of the rate of disappearance of glucose, the extent of which may vary with experimental conditions.

To sum up: the injection or infusion of insulin caused no immediate increase in the apparent glucose space in the normal dog. However, in each of the five experiments, a substantial increase was found 1 hr. or less after the injection, or after the start of the infusion of insulin.

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Effect of Vitamin A on the Cystine Content in Skin of Rats

This investigation was undertaken to gain more precise knowledge of the effect of vitamin A on the epithelium. Diets ranging from a small dose to a liberal supply of vitamin A were fed to rats, and the cystine content of their skin was determined.

The analytical results show that the skin of rats on a diet supplemented with only small doses of vitamin A contained less cystine than that of rats which had been fed with a liberal supply of vitamin A.

The current theory[1] of keratin formation includes oxidation of cysteine sulphydryl groups of adjacent polypeptide chains to produce cystine disulphide linkage, the covalent bonds of the latter grouping serving to hold the keratin molecule together and give it rigidity. Since vitamin A deficiency leads to excessive keratinization of epithelium, the possibility that the vitamin A may be concerned in the metabolism of sulphydryl and disulphide linkages deserves

scrutiny. This observation led to our investigating the change in cystine content in the skin of rats which had been fed diets supplemented with small doses and large doses of vitamin A, with or without sulphur amino-acids.

The test animals used were rats of the Wistar strain of both sexes, those used for the first experiment weighing 30–35 gm., those for the second, 65–70 gm. They were housed in individual compartments with screen bottoms and fed a vitamin A-deficient ration consisting of powdered rice 92, defatted soybean meal 6, sodium chloride 1 and calcium carbonate 1. In addition, each rat was supplied with 20γ thiamine and 50γ riboflavin daily. The food was made into a paste by adding hot water, and cooked. After a three-week depletion period the animals were transferred to the experimental diets. At the end of four weeks the animals were beheaded. After shaving about 5 cm.² of the back of the neck in the area of the shoulder blades, samples of skin were taken from each animal, dried and defatted with ethyl alcohol–ethyl ether. Weighed samples of it were hydrolysed in 3 *N* hydrochloric acid at 120° C. for 4 hr. The cystine content in the hydrolysate was analysed by the phosphotungstic acid method[2].

The cystine content of skin should be affected by the amount of sulphur amino-acid in the diet. In the first experiment, we investigated the effect of the small dose and the effect of the liberal supply of vitamin A in the diet, with or without a supplement of methionine, on the cystine content of the skin. The four groups of rats were given different diets as described in Table 1.

Table 1. Amount of Supplements in Experiment 1

Group No.	1	2	3	4
Vitamin A per rat daily (γ)	1	100	1	100
DL-Methionine (gm.) per 100 gm. of diet	none	none	0·2	0·2

Rats in Group 1 were dosed with only 1γ of vitamin A per rat daily, which is a low dose. Animals in Group 2 were given 100γ of vitamin A—an ample dose. Groups 3 and 4 were dosed with 1γ and 100γ of vitamin A respectively supplemented simultaneously with methionine. During the four-week feeding period, the amount of diet was restricted to 7 gm. per rat daily the first week and thereafter increased each week by 1 gm. a week.

In the second experiment cystine was used instead of methionine and was given in conjunction only with the lower dosage of vitamin A (Table 2).

Table 2. Amount of Supplements in Experiment 2

Group No.	1	2	3
Vitamin A per rat daily (γ)	1	100	1
L-Cystine (gm.) per 100 gm. of diet	none	none	0·2

Table 3. Effect of Vitamin A and Methionine on the Cystine Content of Skin

Values shown with mean and standard deviation of six rats in each group

Group No.	1	2	3	4
Supplement to diet	1γ vit. A	100γ vit. A	1γ vit. A + methionine	100γ vit. A + methionine
Body-weight (gm.):				
Initial	36·3 ±2·13	36·5 ±2·50	36·5 ±2·50	36·5 ±3·10
Gain per day	1·86±0·098	1·96±0·120	1·94±0·070	1·98±0·102
Skin: cystine (per cent)	1·36±0·083	1·60±0·102	1·50±0·132	1·68±0·220

Results of the first experiment show that the cystine content of skin of rats dosed with only 1γ of vitamin A is lower than that of rats given a liberal allowance of vitamin A. In the presence of methionine, the cystine content increased both with small and large doses of vitamin A; however, rats with the ample supply of vitamin A showed a higher content of cystine.

The results of the second experiment were essentially similar to those of the first experiment (Table 4).

Table 4. Effect of Vitamin A and Cystine on the Cystine Content of Skin

Values shown with mean and standard deviation of six rats in each group

Group No.	1	2	3
Supplement to diet	1γ vit. A.	100γ vit. A	1γ vit. A + cystine
Body-weight (gm.):			
Initial	74·8 ±7·54	74·6 ±6·36	74·6 ±8·31
Gain per day	1·89±0·172	2·04±0·189	2·00±0·269
Skin: cystine (per cent)	1·18±0·135	1·53±0·082	1·41±0·205

The cystine content was lower in group 1 and higher in groups 2 and 3, which received an ample supply of vitamin A, and cystine with small doses of vitamin A respectively. The results were in good agreement with the first experiment.

Abdulnabi and Bieri have studied the distribution pattern of amino-acids in the testes and livers and the blood serum of both vitamin A-deficient and normal rats. Paper chromatography did not reveal any difference between the two groups, and the quantitative picture, as obtained by microbiological assay, in general supported the qualitative results. There were, however, three quantitative differences. In the testes of the deficient rats, there was more tyrosine and phenylalanine, whereas in the normal livers there was more threonine than in the livers from the deficient rats.

Our results show that in the skin of the vitamin A-deficient rats there was less cystine than in that of normal rats, suggesting that vitamin A may be involved in the metabolism of cystine and may have an effect on the resistance of epithelium.

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BIOLOGY

A Method of staining Sectioned Tissues with Lead for Electron Microscopy

Solutions of lead are now widely used by some electron microscopists for the production of a general increase in the electron density of cellular constituents in sections of biological material fixed in osmium tetroxide and afterwards embedded in methacrylate or 'Araldite'. Grids bearing the sectioned specimens may be floated face downwards for a few minutes on the surface of weakly ionized lead solutions, for example, lead acetate or 'alkaline' lead hydroxide[1]. During this exposure lead ions become attached to the osmium already deposited on the material during fixation, with a consequent improvement of specimen contrast in the electron beam.

The effective application of these methods of lead impregnation has been somewhat curtailed by the fact that on exposure to air all solutions of lead salts become clouded by the formation of lead carbonate, the insoluble crystals of which are frequently encountered on the surface of sections stained by this method. In order to reduce this contamination

attempts can be made to stain the specimens in a nitrogen or carbon dioxide-free atmosphere[2]; but these measures do not entirely eliminate the nuisance.

Successful lead staining without serious disadvantages can be effected as follows: Add 100 c.c. of distilled water to 1 gm. lead hydroxide and bring to the boil. Cool slightly and filter. To the filtrate, which is faintly clouded, add a solution of 2 *N* potassium hydroxide drop by drop until on agitation a completely clear solution remains. This is the staining fluid and probably contains some lead in the form of a plumbite, thus:

$$2KOH + Pb(OH)_2 = K_2PbO_2 + 2H_2O$$

Float the grids face down on a few drops of this solution in a covered watch-glass for 5 min. Then with a pipette replace the staining solution with distilled water and proceed at leisure to remove the grids individually with forceps. Agitate each grid for some 3–4 sec. (only) in a 1 per cent solution of potassium hydroxide and then immediately dip into a large volume of distilled water. Finally blot the grids on to filter paper and allow them to dry. Some experience is required to gauge just the right amount of time for the cleaning of the specimens in the 1 per cent potassium hydroxide (after staining). If this period is inadequate the grids show some contamination by crystals which are, however, readily removed by further immersion in potassium hydroxide. On the other hand, it has been observed that if lavage in the alkali is too prolonged then lead impregnation of the specimen appears to be reduced.

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5-Hydroxytryptamine in Coelenterates

In an earlier report[1], it was concluded from evidence obtained by pharmacological tests and paper chromatography that 5-hydroxytryptamine was a constituent of the sea-anemones, *Metridium senile* and *Calliactis parasitica*. More 5-hydroxytryptamine-like material was obtainable from *Metridium* tentacles, with their nematocysts, than from body wall which is relatively free of nematocysts. This led to the suggestion that 5-hydroxytryptamine might be a pain-producing factor in these stinging organelles. An independent study[2] of material released on discharge of isolated *Metridium* nematocysts yielded two hydroxyindoles tentatively identified by chromatography as 5-hydroxytryptophan and bufotenine. The identification of 5-hydroxytryptamine in *Calliactis* has now been made quite certain[3,4]. However, the very large amount found in the coelenteric organ of this species and the relatively small amount in tentacles, coupled with the finding of little 5-hydroxytryptamine in *Metridium* and *Anemonia sulcata*, led Mathias, Ross and Schachter[3,4] to conclude that it is unlikely that 5-hydroxytryptamine is associated with nematocysts. Further evidence is now available that this conclusion may not be fully warranted.

With a highly sensitive fluorescence method for the estimation of 5-hydroxytryptamine now available[5], as well as a suitable method for extracting it from tissues[6], it has been possible to confirm and extend our earlier observations on the distribution of a substance like 5-hydroxytryptamine in different parts

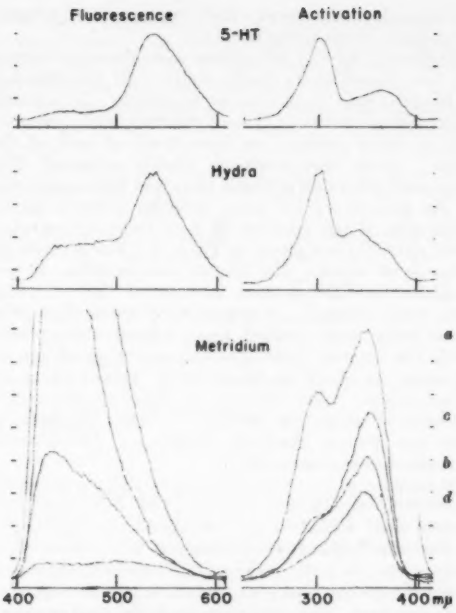

Fig. 1. Fluorescence and activation spectra: 5-HT, 0·1 γgm. 5-hydroxytryptamine/ml. 3 *N* hydrochloric acid; *Hydra*, extract of 0·38 gm. *Hydra oligactis* in 4·2 ml. 3 *N* hydrochloric acid. *Metridium*, (*a*) 0·24 gm. acontia; (*b*) 4·1 gm. body wall; (*c*) 2·3 gm. tentacles (each tissue extracted into 4·5 ml. 3 *N* hydrochloric acid); (*d*) reagent blank

of *Metridium*. The content of 5-hydroxytryptamine of certain other coelenterates has been estimated by this method.

In the presence of 3 *N* hydrochloric acid, there is a fluorescence maximum for 5-hydroxytryptamine at about 540 mμ when activated at 300 mμ (Fig. 1). These are the observed values obtained with the particular spectrofluorometer at our disposal. Bufotenine has a similar fluorescence to that of 5-hydroxytryptamine, but of the coelenterate extracts that we have examined chromatographically only those from *Physalia* tentacles gave an indole spot with an R_F value corresponding to that of bufotenine.

An extract of whole brown *Hydra* contained little interfering substance and gave obvious fluorescence and activation peaks (Fig. 1). Estimations of 5-hydroxytryptamine in one lot of *Hydra* and two lots of *Sagartia*, a small sea anemone, are given in Table 1. Extracts of *Metridium* tissues were found to contain a substance that fluoresces strongly near 430 mμ, thereby obscuring the 540-mμ peak of 5-hydroxytryptamine. Activation peaks were easily

Table 1. 5-Hydroxytryptamine in Coelenterates

Species and part	5-Hydroxytryptamine equivalents μgm./gm. fresh tissue
Hydra oligactis	
Approximately 1,000 whole animals	1·5
Sagartia luciae	
15 whole animals per determination	0·74 1·3
Metridium senile	
Whole animals*	0·04; 0·05; 0·05
Body wall	0·02; 0·05; 0·09
Oral disk and tentacles	0·08; 0·12; 0·17
Tentacles	0·24; 0·47
Acontia	0·60; 0·97; 1·30

* Three extractions and estimations made on the same day on animals maintained in laboratory for several weeks.

distinguished, however, and quantitative estimates were made from them (Fig. 1).

Different parts of *Metridium* were chosen to contain: (1) few nematocysts (body wall); (2) an abundance of nematocysts (tentacles or acontia); (3) a number intermediate between these (oral disk with tentacles). Two or three extractions were made of each of these parts. Some were made on freshly collected *Metridium* and others on animals that had been maintained in the laboratory for some months without feeding. Estimates of the content of 5-hydroxytryptamine of these extracts are given in Table 1. For a given part a range of values was found, attributable, in some measure, to the previous history of the individuals that were utilized. However, it is clear that *Metridium* body wall yielded least 5-hydroxytryptamine while the acontia, rich in nematocysts, yielded nearly 20 times as much as body wall. Other parts were intermediate. As a result of these findings, I am inclined to state, as earlier[1]: "This suggests, but does not prove, that the 5-HT (of *Metridium*) is contained in nematocysts . . .".

Mathias *et al.*[4] say that of several coelenterates they examined, significant amounts of 5-hydroxytryptamine were found only in *Calliactis parasitica*, and by far the highest concentration in "tissues of the coelenteric cavity". Thus far, 5-hydroxytryptamine in invertebrates has been found in venom apparatus and venoms[7], also nervous systems and excretory organs[8]. It would be of much interest to determine in which coelenteric tissue of *Calliactis* the 5-hydroxytryptamine is located. In the meantime it seems that the distribution of 5-hydroxytryptamine in *Metridium* is related to the distribution of nematocysts; but no claim is made that this is a specific (restricted) relationship.

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Selective Elimination of Single Sense Cells with Methylene Blue

For many problems of epigenetics and physiology it is desirable to destroy selectively individual cells within a tissue complex in order to test their functions. Although, in favourable cases, such cell structures as the nucleus have been eliminated, whole-cell destruction often implies damage to the surrounding tissue. Another difficulty of such experiments is the fact that recognition of single living cells within the tissue may be impossible. The use of toxic substances of sufficient specificity to affect the cell type to be eliminated, but without effect on other cells or on the organism as a whole, would solve this problem. A possible avenue of approach seems to be through techniques involving vital or supravital dyestuffs. The actual method must, of course, depend upon the type of cell to be eliminated. The experiments to be described illustrate this method of selective cell elimination by means of a chemical. The cells to be eliminated were single sense cells.

Young pupæ of the wax moth, *Galleria mellonella* L., were used in this work. Ether narcosis of half-hour duration served to immobilize the animals for several hours. Crystalline methylene blue is introduced with the point of a fine iridectomy knife. It dissolves at once in the hæmolymph, which takes a dark blue stain. Through carefully applied pressure, the blue hæmolymph is now distributed in the region to be studied. After ½–1 hr., the sense cells and the nerves are stained and they are now perceptible *in vivo* through the thin cuticle. Generally the nerves are less intensely stained than the sense cells. Body movements of the animals after recovering from narcosis distributed the dyestuff more evenly. After some time all dye will be reduced to its leuco form. Practically all animals survive the operation without showing any defect.

Study of slides from fixed material shows the stain exclusively located in the sense cells, disregarding those cells at the wound which were damaged by the operation. Some of the sense cells are stained light blue, others are dark blue. This difference in staining intensity is shown even by the sense cells of the same sensillum. According to statements in the literature[1], it is to be expected that at least the dark blue cells are destroyed. This is confirmed by histological study. Fig. 1 shows groups of cells

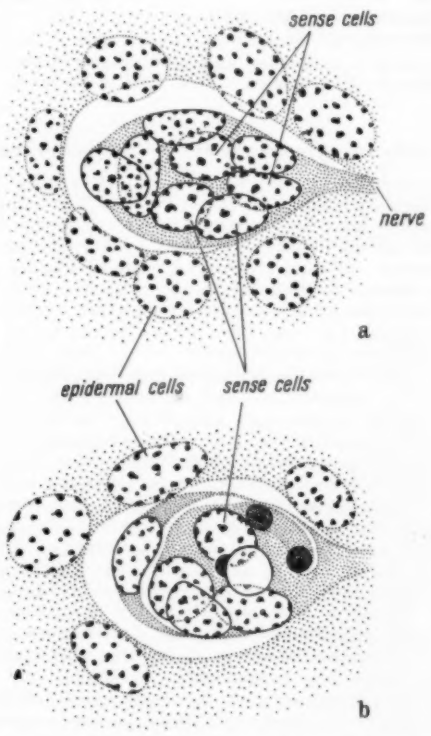

Fig. 1. Cell groups which belong to sensory bristles on the wing of *Galleria*. *a*, A normal cell group; *b*, a cell group in which three sense cells are destroyed by methylene blue. Nuclei which do not belong to the bristle have dotted lines. Pupæ, 25 hr., Bouin-Allan fixed, stained with hæmatoxylin

belonging to sensory bristles on the wing before outgrowth of the bristles themselves. The normal cell complement of such bristles (Fig. 1*a*) consists of eight cells, four of which are sense cells. The others are the trichogen and the tormogen cell and two cells of still unknown function, called *Hilfszellen*[2]. In the group of cells shown in Fig. 1*b*, three sense cells are damaged by methylene blue; two nuclei are pycnotic, one is swollen up like a blister, with clotted chromatin. This pupa was fixed for about 20 hr. after treatment with methylene blue. In animals which were fixed later the sense cells are completely decayed. The fourth sense cell and the four other cells in Fig. 1*b* are not damaged. Such cell groups as in Fig. 1*b* perform their normal function, that is, they give rise to entirely normal bristles, only with a reduced number of sense cells. This indicates that they are physiologically normal.

According to our experience certain prerequisites are necessary whenever a method of selective cell elimination through the use of specific substances is to be applied. (1) It must be possible to localize the toxic substance and to maintain a high concentration for a period of time in the region under study (narcosis; sack-like organs, see below). (2) The substance should be easily distributed (spongy tissue, much hæmolymph). (3) The substance should be implanted in as pure a state as possible, for example, not dissolved in another fluid. Conditions 1 and 2 were fulfilled, in the young pupa, by the extremities, the antennæ and the wings.

By use of the method described it was possible to show that the sense cells exert an influence on the development of the sensilla in the integument of *Galleria*. If this influence is lacking, polyploidization of the trichogen and the tormogen cells does not occur. These results will be reported in detail elsewhere[3].

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A Spring Oat Variety resistant to the Stem Eelworm

RESISTANCE among oat varieties to the stem eelworm, *Ditylenchus dipsaci* (Kühn) Filipjev, was first shown by Goodey[1], who recorded a high degree of resistance in the varieties Grey Winter and Aberystwyth *S*81. Since this record, resistance has been recorded among commercially grown varieties, in Milford (*S*225)[2], in *S*172, Early Grey Winter and Unique[3]. The following is a record of resistance in a new spring oat variety.

An oat variety trial in Denbighshire, conducted jointly by the National Agricultural Advisory Service and the National Institute of Agricultural Botany, had been sown inadvertently in the early spring of 1959 in a field which was later found to be infested with the oat race of stem eelworm. In the trial the varieties were sown in single drill widths 140 yards long in a randomized block lay-out with four-fold replication. Damage by stem eelworm occurred in five of the varieties—Sun II, Civena, Blanche de Wattines, Condor and *V*2661. The remaining variety, number 4757/18/2/19, a form bred by the Welsh Plant Breeding Station, Aberystwyth, showed, apart from a few isolated plants, a high degree of resistance.

The Welsh Plant Breeding Station cereal breeding department gives the pedigree of the resistant variety as a selection from a cross between 01750/11, a mildew-resistant selection developed at the station, and Tama, a crown rust-resistant selection developed at Iowa State College. The variety is to be introduced commercially under the name of Manod (*S*235).

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Foliar Abnormalities, including Repeated Branching and Root Formation, induced by Kinetin in Attached Leaves of *Marsilea*

THE leaf of the water fern *Marsilea* is normally a strictly determinate structure, and even the supply of a wide range of physiologically active substances, to sporelings growing in aseptic culture, has hitherto resulted in no fundamental changes in leaf morphology[1,2]. Some interesting leaf abnormalities have now been obtained, however, following the addition to the culture medium of the cell-division stimulating substance, kinetin[3].

In the investigations outlined here, sporelings of *Marsilea drummondii* were grown aseptically in a liquid medium, consisting of a Knop's solution supplemented by glucose (analytical reagent) at concentrations of either 2 or 4 per cent. Kinetin solutions were added aseptically at various early stages of sporeling development. Resulting changes in leaf morphology were so unusual that the experiments were repeated with several batches of cultures, but all yielded essentially similar results.

In the initial experiment, kinetin was supplied to 10-day old sporelings, at concentrations of 10, 1·0 and 0·1 mgm./litre. The two higher concentrations were toxic, while at 0·1 mgm./litre sporeling development continued, but was markedly abnormal, the new growth consisting of an irregular mass of repeatedly branched structures, some of which were undoubtedly modified leaves. In later experiments, kinetin was added to give concentrations of 0·05, 0·01 and 0·002 mgm./litre. At 0·05 mgm./litre the results were virtually identical with those obtained at 0·1 mgm./litre, but at the two lower concentrations of kinetin less-extreme abnormalities were observed.

In the culture medium containing 0·002 mgm./litre kinetin, development of many of the sporelings was apparently completely normal; but in a proportion of the sporelings leaf development was somewhat affected. Some sporelings produced acicular, non-laminate leaves for a period, before reverting again to normal development, while other sporelings bore leaves with shorter petioles and longer leaf lobes than the corresponding structures of normal leaves.

The most interesting leaf abnormalities were obtained with 0·01 mgm./litre of kinetin. Most sporelings at first produced relatively normal leaves,

though with short petioles. The first marked deviation from normal development was the production of several roots from the adaxial surface of some of these leaves, near the junction of lamina and petiole (Fig. 1*e*). The outgrowth of roots from *Marsilea* leaves has not been observed previously, even after the addition of so powerful a root-inducer as 2,4-D [1]. Root formation was usually followed by the appearance of a meristematic outgrowth, which in turn gave rise to a series of abnormal leaves (Fig. 1*d*). After longer exposure to the kinetin solutions the sporelings developed even more striking leaves, showing repeated branching of the leaf segments (Figs. 1*a*, *b*, *c*).

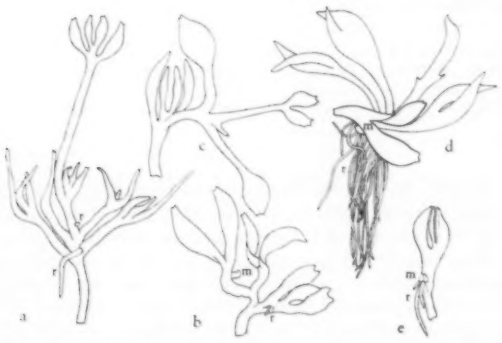

Fig. 1. *Marsilea drummondii* A.Br. Abnormal leaves from sporelings grown in a medium containing 0·01 mgm./litre of kinetin. *m*, Meristem; *r*, roots or root. (All × *c*. 2)

The leaf abnormalities briefly described above would appear to be of both phylogenetic and developmental interest. On the basis of comparative morphological studies, it is apparently generally accepted (for example, Bower[4]) that the strictly quadrifoliate adult leaf of *Marsilea* is derived from an ancestral type with more elaborate structure. The present work indicates that the potentialities of more extended development are still inherent in the *Marsilea* leaf.

Apart from its toxic action at high concentrations, the effects of kinetin appear to be restricted to meristematic tissues. In the present work, kinetin at physiological concentrations had no evident effect on fully developed leaves of *Marsilea* sporelings or on excised leaves of the same cultures. Our results suggest that the limited growth of the leaf meristems of normal plants of *Marsilea* may be due to correlations with other parts of the plant involving a restriction in the supply of cell-division promoting substances similar to kinetin. In any event, the external supply of kinetin disturbs the normal correlations and permits a more prolonged activity of the leaf meristems.

It is hoped to publish further details of this work, including anatomical observations, in a later contribution.

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A Comparison of Adhering and Abscising Fruits of Peach treated with N-1-Naphthyl Phthalamic Acid

The search for a thinning spray to be used on peaches has shown that N-1-naphthyl phthalamic acid promotes abscission of peach fruits[1]. This preliminary report compares the size, content of natural auxin, and embryological development of adhering and abscising fruits of Halehaven peach trees sprayed with N-1-naphthyl phthalamic acid at petal fall.

On May 13, 1958, Halehaven peach trees at early petal-fall stage were sprayed with 175 p.p.m. of N-1-naphthyl phthalamic acid. By May 29 heavy fruit drop had started on the sprayed trees. The dropping fruits were comparable in size to those which fell from unsprayed trees a month later. This indicates that N-1-naphthyl phthalamic acid hastens drop.

150 tightly adhering fruits and 150 very loosely adhering fruits (here called abscising) were picked on May 29 from one tree sprayed with N-1-naphthyl phthalamic acid. The loosely adhering fruits probably would have fallen within 24 or 48 hr. had they been left to drop naturally. No differences in colour of the adhering and abscising fruits were observed.

Volume measurements were made on the fruits by water displacement. The ovules were then removed and physical measurements made on twenty of them. Differences in fruit volume, ovule length and weights between adhering and abscising material were not great, but in every category the adhering fruits were larger (Table 1).

Table 1. Comparison between Adhering and Abscising Fruits of Halehaven Peach Trees sprayed with N-1-Naphthyl Phthalamic Acid

	Adhering	Abscising
Volume per fruit (ml.)*	0·22	0·20
Ovule length (mm.)	3·92	3·50
Fresh weight per ovule (mgm.)	6·92	5·85
Dry weight per ovule (mgm.)	0·93	0·91
Indoleacetic acid equivalent (μgm./ovule)	0·00221	0·00031
Indoleacetic acid equivalent (μgm./gm. dry weight)	2·38	0·34
Mean number of cells per embryo	11·1	6·3
Mean number of endosperm nuclei per ovule	155	99

* Volume per fruit at bloom was < 0·22 ml.

Ovules were frozen on dry ice, lyophilized, and bioassayed for auxins using silica-gel column chromatography[2] and the *Avena* first-internode test[3]. Preliminary work with extracts of ovules collected two weeks after bloom from unsprayed Halehaven trees indicated that two growth-promoting substances could be separated. One of very low concentration was eluted in a position similar to ethyl indoleacetate, and another of somewhat higher concentration came out in the indoleacetic acid position (unpublished results). A single determination of extracts of ovules from sprayed trees and subsequent bioassay of the eluted fractions corresponding to the ethyl indoleacetate and indoleacetic acid positions were made. Ovules from both adhering and abscising fruits contained only traces of the growth-promoting substance eluted in the ethyl indoleacetate position, but there was about seven times as much of the substance similar to indoleacetic acid in the ovules from adhering fruits as from those from abscising fruits (Table 1).

Ovules were fixed in a formalin–acetic acid–ethyl alcohol (85:5:5 per cent) mixture, embedded in

paraffin, sectioned at 15 or 20μ, and stained in Heidenhain's hæmatoxylin. In the nine adhering and the thirteen abscising ovules studied, embryos of about 1–23 cells and free nuclear endosperm of about 5–301 nuclei were found. As shown in Table 1, the average number of cells in the embryo and the number of endosperm nuclei of abscising ovules were about 60 per cent of those in the adhering ovules, although these means were not significantly different at the 5 per cent level of probability. In most of the abscising ovules there was more shrinkage of cytoplasm, and less differential staining of many nuclei in embryo and endosperm than in the adhering ovules. In about half the abscising ovules the embryo stained darkly. These changes in staining reaction are interpreted, on the basis of other work on seed abortion, as indicating the beginning of the breakdown of the tissues.

It appears that N-1-naphthyl phthalamic acid causes ovule abortion and abscission in peaches in a manner similar to the action of naphthaleneacetic acid on apples[4]. Whether the primary effect of N-1-naphthyl phthalamic acid is on the endosperm, embryo or some other tissue is still to be determined. While little difference in the size of the fruits and ovules was found in these lots of adhering and abscising fruits treated with N-1-naphthyl phthalamic acid, larger differences were found in the auxin content and in the histological appearance of the embryo and endosperm of each lot of ovules (Table 1). The difference in auxin content of adhering and abscising ovules corresponds to Luckwill's report of lower auxin content in naturally dropping fruits of apples than in adhering fruits[5].

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Presence of Gibberellin-like Substances in Lettuce Seed

Gibberellins and gibberellin-like substances are being shown to occur in an increasing number of higher plants. Immature seeds of numerous species contain particularly large amounts of these substances[1], but the levels in mature seeds of the same species are much lower[2]. Certain seeds respond very strongly to applied gibberellin, this being particularly evident in certain 'light-requiring' seeds, for example, those of lettuce[3,4] and *Arabidopsis thaliana*[5], in which gibberellin treatment may result in complete germination in the dark. The obvious first step towards understanding the physiological significance of this

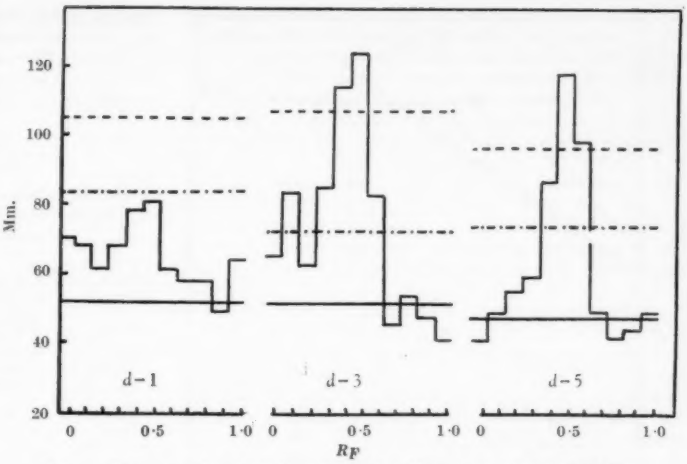

Fig. 1. Histogram of extracts from dry seeds of lettuce, tested for gibberellin-like activity on *d*-1, *d*-3 and *d*-5 dwarf maize. Each test plant was treated with a quantity of extract equivalent to 50 gm. of dry seed. Abscissa, R_F values; ordinate, sum of lengths of first and second leaf sheaths of test plants. ———, Untreated controls; –·–·–·–, 0·01 μgm. gibberellin A_3; – – – –, 0·1 μgm. gibberellin A_3. R_F value of gibberellin A_3, 0·6

gibberellin action is to determine whether gibberellin-responsive seeds contain endogenous, gibberellin-like growth regulators. The present communication reports on such studies with mature seeds of lettuce (*Lactuca sativa* L.).

The seed used was of the Grand Rapids variety, obtained locally and possessing a pronounced light requirement (at 26°, dark germination about 10 per cent; germination after saturation irradiation with white or red light 95–100 per cent).

The extraction method was that of West and Phinney[6] with some minor modifications; it is largely identical with that used by one of us[7] in similar work with vegetative and photoperiodically induced plants of *Hyoscyamus niger*.

The assay was performed on three monogenic dwarf mutants of maize (*Zea mays* L.), *d*-1, *d*-3 and *d*-5 (ref. 9). The use of these mutants as assay material is essential since their response is highly specific for gibberellins and furthermore permits the recognition not only of quantitative, but also of qualitative, differences in the activities of different gibberellins and gibberellin-like materials. Thus, gibberellins A_1, A_2 and A_3 and numerous gibberellin-like materials from various plants are highly active on all three mutants, but another gibberellin-like material, bean-factor II (ref. 6), probably identical with gibberellin A_5 (ref. 10), while highly active on *d*-3 and *d*-5, is only 5 per cent as active as gibberellin A_3 on *d*-1.

Altogether, three experiments were performed, giving highly consistent results. A representative histogram is shown in Fig. 1. It is evident that there is a zone of pronounced gibberellin-like activity in the R_F region of 0·3–0·6, with a maximum at 0·4–0·5. Furthermore, this activity is much more pronounced on the *d*-3 and *d*-5 mutants than in the *d*-1 test; thus the active material in the extracts shows the activity pattern of bean factor II and not of gibberellins A_1, A_2 and A_3. Some indications of activity were also found in other regions of the chromatograms (for example, at $R_F = 0{\cdot}1$ on the *d*-3 mutant in Fig. 1); these, however, varied between experiments and thus require further study.

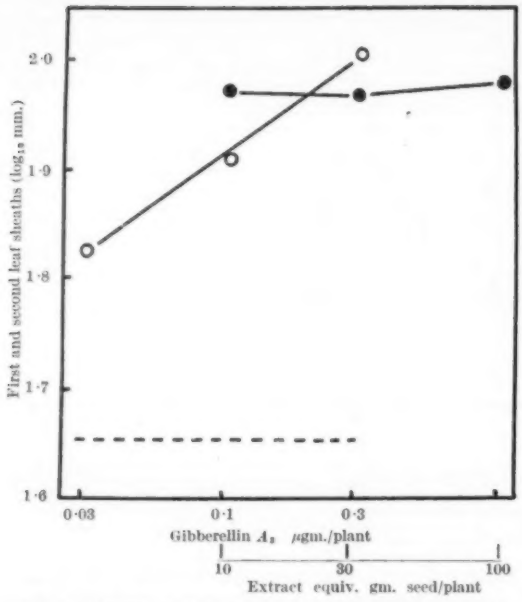

Fig. 2. Growth response of *d*-3 maize seedlings treated with different amounts of gibberellin A_3 (○——○) and of extract from dry lettuce seed (●——●). Broken line, untreated controls

Fig. 2 shows an attempt at a quantitative estimation of the amount of active material. For this experiment, the active zones of the chromatogram shown in Fig. 1 were re-extracted with acetone and the response of *d*-3 mutant seedlings to this extract was compared with their response to different amounts of gibberellin A_3. The response of the seedlings to 0·03 μgm. to 0·3 μgm. of gibberellin A_3 per plant, when plotted as logarithm of growth against the logarithm of dose, follows a straight line, as is characteristic of the dwarf-maize assay in the range of approximately 0·003–3·0 μgm. gibberellin per plant[8]. However, varying amounts of extract, corresponding to 10, 30 and 100 gm. of the original seed weight, all produced responses of practically equal magnitude. This fact indicates that the extracts still contained materials which, with increasing amounts, reduced the response of the plants. Therefore, precise estimations of the quantity of active material are not yet feasible; however, it can be assumed from Fig. 2 that the minimum level is in the order of 0·1–0·3 μgm. per 10 gm. of seeds or 10–30 μgm. per kgm., expressed as equivalents to gibberellin A_3.

It is thus clear that dry lettuce seeds contain gibberellin-like, endogenous growth substances which are physiologically related to bean-factor II and the quantities of which seem to surpass considerably the values reported for other mature seeds[9], although they still seem to be below the levels of activity found in immature seeds.

Gibberellin-like activity was also found in imbibed seeds. It is planned to pursue this matter further, and in particular, to study the gibberellin content in relation to germination.

This work was performed while one of us (A. L.) was on sabbatical leave from the University of California, Los Angeles, and holder of a senior post-doctoral fellowship from the National Science Foundation, Washington, D.C. He wishes to thank the staff of the Hebrew University's Botany Department and its chairman, Prof. M. Evenari, for their hospitality. We are greatly indebted to Dr. B. O. Phinney, University of California, Los Angeles, for supplying seeds of the three maize mutants.

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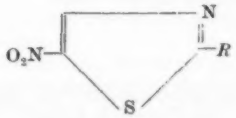
MICROBIOLOGY

Specific Inhibition of Obligate Anaerobes

Many drugs are known which optimally inhibit single groups of micro-organisms (bacteria, fungi, or protozoa), or which inhibit specific types within one of these groups (Gram-positive or Gram-negative bacteria, pathogenic or non-pathogenic fungi). For example, certain antibiotics only inhibit bacteria; the polyene antibiotics only inhibit fungi; fumagillin inhibits protozoa[1]. This list can be extended to include compounds other than antibiotics: crystal violet (Gram-positive bacteria), sodium azide (Gram-negative bacteria). These compounds, possessing taxonomic specificity, are clearly contrasted with non-specific antimicrobial agents such as the halogens, phenols, and quaternary ammonium compounds.

I am concerned here with an antimicrobial activity which appears to transcend major taxonomic boundaries in an unusual fashion. Compounds of the type,



where R is a substituted amino group, are selective inhibitors of obligate anaerobes. In particular, I wish to report on the activity of an acetylated piperazine derivative, 1-(5-nitro-2-thiazolyl)-4-acetyl piperazine[1]. Susceptible anaerobes include Gram-positive and Gram-negative bacteria, actinomycetes, and protozoa, but not their aerobic counterparts. This activity may be regarded as a special kind of physiological specificity, which does not appear to have been reported.

All determinations of antimicrobial activity were made under growth conditions optimal for the organ-

Table 1. MINIMUM CONCENTRATIONS OF 1-(5-NITRO-2-THIAZOLYL)-4-ACETYL PIPERAZINE TO INHIBIT THE GROWTH OF ANAEROBIC AND AEROBIC MICRO-ORGANISMS

Anaerobic organism	μgm./ml.	Aerobic organism	μgm./ml.
Trichomonas vaginalis (strain 1)	0·7–1*	*Euglena gracilis*	>1,000
Trichomonas vaginalis (strain *H*)	0·7–1	*Trypanosoma avium*	>1,000
Trichomonas vaginalis (strain *J*)	0·7–1	*Physarum polycephalum*	333
Trichomonas foetus (strain *BR*)	0·7–1	*Lactobacillus casei*	>1,000
Endamoeba histolytica	12–25	*Escherichia coli*†	1,000
Clostridium novyi	1–4	*Proteus vulgaris*	>1,000
Clostridium sporogenes	1–4	*Salmonella typhosa*	333
Clostridium tetani	1–4	*Pseudomonas aeruginosa*	>1,000
Clostridium perfringens	250	*Neisseria catarrhalis*	>1,000
Fusobacterium sp. (human)	1–4	*Staphylococcus aureus*	>1,000
Fusobacterium sp. (guinea pig)	1–4	*Streptococcus faecalis*	>1,000
Fusobacterium sp. (rabbit)	1–4	*Streptococcus pyogenes*	>1,000
Veillonella gazogenes	8–16	*Leptospira icterohaemorrhagiae*	1,000
Desulphovibrio desulphuricans	1–4	*Candida albicans*	>1,000
Spherophorous necrophorous	1–4	*Trichophyton mentagrophytes*	>1,000
Actinomyces bovis	111	*Streptomyces* sp.	>1,000
Treponema pallidum (Reiter strain)	1–4	HeLa strain (Gey)	1,000

* Figures represent range or average of three determinations.

† Engel's porphyrinless mutant of this parent strain sensitive at 111 μgm./ml. (see text).

ism studied. Anaerobic bacteria and *Lactobacillus casei* were grown in Brewer's thioglycollate broth ('Difco') supplemented with 10 per cent of rabbit serum when necessary; trichomonads in Kupferberg's simplified trypticase serum broth; trypanosomes in Diamond's blood broth[3]; *Euglena gracilis* under artificial illumination in acetate–peptone–yeast extract broth; *Desulphovibrio desulphuricans* in Van Delden's broth[4]; and *Leptospira icterohaemorrhagiae* in a semi-solid beef extract–rabbit serum medium. The slime mould *Physarum polycephalum*, maintained in the plasmodial 'animal' phase, was grown on 1·0 per cent oat-meal agar. Malignant human epithelial cells, strain HeLa (Gey), were grown on glass in Hank's balanced salt solution, *p*H 7·0, supplemented with lactalbumin hydrolysate (0·5 per cent), yeast extract (0·1 per cent), calf serum (10 per cent), penicillin (50 units per ml.), and streptomycin (50 μgm. per ml.).

The activity of the piperazine drug is shown in Table 1. The aerobic and facultative organisms were approximately 300–1,000 times more resistant to the drug than the obligate anaerobes, regardless of taxonomic status. When inhibition of an aerobe did occur, as with *Physarum*, *Salmonella*, *Escherichia*, *Leptospira* and strain HeLa cells, a relatively high concentration of drug was required. It is difficult, of course, to state where sensitivity ends and resistance begins, but the broad outline of aerobic resistance and anaerobic sensitivity is apparent. The anomalous response of our strain of *Clostridium perfringens* (250 μgm./ml. for inhibition) suggests further experiments to determine if shifts of susceptibility can occur in a continuously exposed population.

The effect of 1-(5-nitro-2-thiazolyl)-4-acetyl piperazine is microbicidal, since exposed populations are rendered sterile within 24 hr. at the minimum inhibitory concentrations stated. Activity against susceptible anaerobes is independent of *p*H, exogenous protein, and cannot be reversed by twenty times the concentration (on a molar basis) of thiamine, riboflavin, biotin, calcium-D-pantothenate, nicotinamide, pyridoxamine, folic acid, inositol, guanine, adenine, xanthine, hypoxanthine, thymine, or uracil.

An experiment was conducted to determine if the selective inhibition of obligate anaerobes was related to anaerobiosis *per se*, that is, to a sensitivity potentiated by the environment. If this were so, anaerobic growth of the facultative organisms studied (*Staphylococcus aureus*, *Streptococcus faecalis*, *Escherichia coli*, *Shigella dysenteriae*, and *Candida albicans*) might induce sensitivity to this drug. This was not the case. These organisms, grown anaerobically in nutrient broth (alkaline pyrogallol technique), displayed their usual resistance to the piperazine drug.

As indicated in Table 1, a porphyrinless mutant of *Escherichia coli*[5], kindly supplied by Dr. M. S. Engel, of the Oak Ridge National Laboratory, was examined for susceptibility to the piperazine drug. The mutant, capable of growth and respiration with or without exogenous hæm, was ten times more sensitive to this drug than the parent strain. With another member of the nitrothiazole series (R = acetamido) the difference was more striking: inhibition of the parent strain at 250 μgm. per ml., total inhibition of the mutant at less than 1·0 μgm./ml.

An attempt was made to induce sensitivity in the above-mentioned facultative organisms by blocking hæm pigments with potassium cyanide. When these organisms were grown in the presence of the highest concentration of cyanide compatible with growth ($2–10 \times 10^{-3}$ M at *p*H 7·2), the usual resistance to the piperazine drug was encountered. Therefore, chemical inactivation of hæm was not equivalent to loss of hæm by mutation.

It should be pointed out that *Desulphovibrio desulphuricans*, which possesses a reversibly oxidizable pigment[6] similar to cytochrome c_3, was as sensitive as anaerobes totally devoid of such pigments (for example, *Clostridium novyi*, *Trichomonas vaginalis*). There is apparently something more fundamental to poisoning by 1-(5-nitro-2-thiazolyl)-4 acetyl piperazine (and perhaps to anaerobiosis) than mere absence or presence of hæm.

In addition to this unique *in vitro* activity, the piperazine drug was effective as an oral trichomonacide in mice. Albino males, carefully screened to weigh 12–15 gm., were infected intraperitoneally with 3×10^6 cells from a 48-hr. culture of *Trichomonas foetus*. Untreated control mice showed 100 per cent mortality usually within 10 days (severe abdominal distension with marked peritonitis). Mice treated once orally with 80 mgm./kgm. of the piperazine drug (3½ min. after infection) showed 100 per cent survival. The acute oral *LD*50 in mice was 1,200 mgm./kgm.; the 50 per cent curative dose (*CD*50) for orally treated mice was about 48 mgm./kgm. of body-weight, as calculated in a dose–response study by the method of Litchfield and Wilcoxin[7]. Further chemotherapeutic studies in animals and man seem warranted, not only for trichomoniasis, but also, indeed, for any infection produced by an obligate anaerobe (for example, amœbic, spirochætal, and clostridial infections).

Compounds of the type described should also prove invaluable in studies designed to elucidate the mechanism of anaerobic growth. Extrapolations and excursions into anaerobiosis in mammalian cells may prove interesting.

I wish to thank Dr. David B. Reisner for supplying the nitrothiazolyl piperazine, for suggesting its antimicrobial evaluation, and for his advice and encour-

agement. The technical assistance of Mr. Harry Jones is gratefully acknowledged.

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BACTERIOLOGY

Physiology of D-Glutamic Acid-requiring Mutants of *Bacillus subtilis*

As reported in a previous paper[1], the 3*d* mutant of *Bacillus subtilis K* requires either D-glutamic acid, D-aspartic acid or D-alanine for growth, and the 3*a* mutant either D-glutamic acid, D-aspartic acid, L-glutamic acid or L-aspartic acid. This communication is concerned with the cell physiology of these mutants in a medium devoid of D-glutamic acid.

Three media were employed. Minimal medium (*MM*) was prepared as prescribed by Gray and Tatum (L-asparagine was eliminated). The medium lacking the nitrogen components (ammonium chloride and ammonium nitrate) of Gray and Tatum's prescription was termed the *M–N* medium; and the minimal medium supplemented with 100 μgm./ml. of D-glutamic acid the *DG* medium. Cells were precultivated in the supplemented medium for 15 hr. at 30°, and after centrifugation and washing, each 0·1 ml. of the cell suspension was inoculated into 10 ml. of *MM*, *M–N* and *DG* media. Cultivation was in Monod tubes on a shaking machine at 37°.

Fig. 1 (I) shows growth-curves of strain 3*d*. The number of viable cells in *MM* medium increased for a while and, after a lapse of 7 hr., decreased rapidly. It was supposed that the rapid lysis observed might have been induced by the unbalanced reproduction between cell wall and cytoplasm. If D-glutamic acid were an essential component of the cell wall, the latter would not be reproduced under the existing conditions, while, because the system concerning the biosynthesis of L-glutamic acid ought to be active in this strain, reproduction of cytoplasm would proceed independently. The residual growth observed in an earlier period of cultivation might be due to the free D-glutamic acid present in the inoculum. This assumption is consistent with the results in Fig. 1 (II), which shows the growth-curves of strain 3*a*. It is reasonable that no significant residual growth is observed in *MM* medium, because the biosynthetic pathway of both D- and L-glutamic acids is equally blocked in this strain.

D-Glutamic acid in cell walls was identified as follows: First, washed cells of *Bacillus subtilis K* were ground in a mortar with fine glass beads, and then the cell wall fraction was separated from other parts by differential centrifugation. The sample was washed with sodium chloride solution (1 *M*) and water repeatedly, and 50 mgm. of the dried material was hydrolysed in 5 ml. of 3 *N* hydrochloric acid for 20 hr. at 110°. A small portion of the residue after evaporation was developed on a paper using a mixture of butanol, acetic acid and water (4 : 1 : 2) as solvent. Glutamic acid (D- or L-) was extracted from the corresponding portion of the paper, and added to *MM* medium to test whether strain 3*d* could grow in this supplemented medium. As was expected, the strain grew well in the medium. Meanwhile, no growth of the strain 3*d* was observed in the *MM* medium supplemented with glutamic acid (D- or L-) from protoplasts of the same bacterium. The protoplasts were prepared by treatment with lysozyme. It is concluded, therefore, that a detectable amount of D-glutamic acid is contained in the cell wall of this bacterium but not in the cytoplasm.

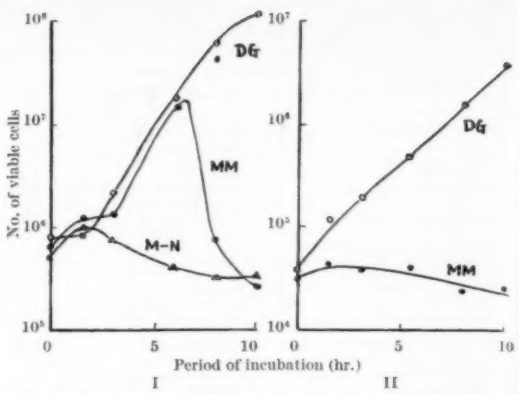

Fig. 1. Growth curves of strains 3*d* (I) and 3*a* (II) in *MM*, *M–N* and *DG* media

The phenomenon observed above is very similar to that found in diaminopimelic acid-requiring mutant of *E. coli*[2], and, probably, to the lysis of *E. coli* by penicillin treatment[3].

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Effects of Chloramphenicol on the Recovery of Ultra-violet-irradiated *Escherichia coli B*

Doudney[1] has recently shown that the addition of chloramphenicol immediately following the ultra-violet irradiation of *Escherichia coli B* prevents the recovery of the bacteria as indicated by the decrease in viable count, whereas such an addition made after 40 min. incubation with or without nitrogen source, like an incubation in nitrogen-free medium, markedly enhances the recovery. On the basis of these and other pertinent observations, Doudney presented the idea that the synthesis of ribonucleic acid and protein, as required for the resumption of deoxyribonucleic acid synthesis following the temporary inhibition thereof caused by ultra-violet light, and the prevention of subsequent unbalanced growth[2] by inhibiting the synthesis of protein and ribonucleic acid are necessary conditions for the recovery of ultra-violet-irradiated bacteria. Experiments of similar nature made by us, however, do not seem to support such a view.

According to Aronson and Spiegelman[3], the lower concentration of chloramphenicol (20 μgm./ml.) as

used by Doudney might be insufficient to block the protein synthesis completely, leading to a different response of bacteria from that observed with higher concentrations of the antibiotic in certain aspects of bacterial physiology. In our experiments a higher concentration (100 μgm./ml.) was employed unless otherwise stated. Further, Doudney used a rather heavy dose of ultra-violet light which reduced the viable count of strain *B* by a factor of more than 10^4. Under such conditions, the recovery of bacteria in a nitrogen-free medium showed a lag of 40 min. or more[1,4]. This agrees with our own observation reported elsewhere[5]. However, even with such a high ultra-violet dose, chloramphenicol added to nitrogen-free medium (0·1 *M* phosphate buffer (*p*H 7·0) – 0·02 *M* glucose) immediately following the irradiation promoted a marked recovery without a noticeable lag, although in *tris*–glucose–salt–casamino acid medium[5] the antibiotic induced the recovery to a smaller extent and with a lag of about 40 min. during which the viable count decreased slightly[5].

When we used a smaller dose of ultra-violet light to yield a survival ratio of around 10^{-3} the recovery in nitrogen-free medium started without delay and chloramphenicol did not significantly affect such a recovery (Fig. 1*B*). Under such conditions of irradiation, chloramphenicol added to *tris*–glucose–salt–casamino acid medium immediately following the irradiation also gave rise to a bacterial recovery without delay, although the recovery was more pronounced when the antibiotic was added after 40 min. incubation in *tris*–glucose–salt–casamino acid medium[5]. That such was not due to the higher concentration of chloramphenicol is illustrated in Fig. 1*A*, in which 20 μgm./ml. of the antibiotic, the concentration employed by Doudney, was also effective in promoting the recovery without lag even when added at 0 min. of incubation. It should be noted that in experiments shown in Fig. 1, the recovery of bacteria in nitrogen-free medium and in growth medium with chloramphenicol followed different kinetics, indicating some difference in the mechanism involved.

The synthesis of deoxyribonucleic acid in our lightly irradiated bacteria showed a lag of about 40 min. in *tris*–glucose–salt–casamino acid medium, while those of ribonucleic acid and protein started without delay, and chloramphenicol added at 0 or 40 min. of incubation, as observed by other workers[1,6] with lower concentrations of the antibiotic, completely inhibited or allowed to a certain extent, respectively, the resumption of deoxyribonucleic acid synthesis.

The discrepancy between Doudney's and our own observations may be due partly or entirely to the slight difference in experimental conditions or in the strain used. However, our experiments clearly indicate that the inhibition by chloramphenicol of the resumption of deoxyribonucleic acid synthesis does not necessarily prevent the recovery of ultra-violet-irradiated bacteria, and this may be sufficient evidence for ruling out the possibility suggested by Doudney[1]. It was also demonstrated[7] that the unbalanced growth could not be taken as the cause of bacterial death either in thymine-deficient, ultra-violet-irradiated, or mustard-treated *E. coli*. Thus, the possible mechanism of the recovery of ultra-violet-irradiated *E. coli B* is still open to question.

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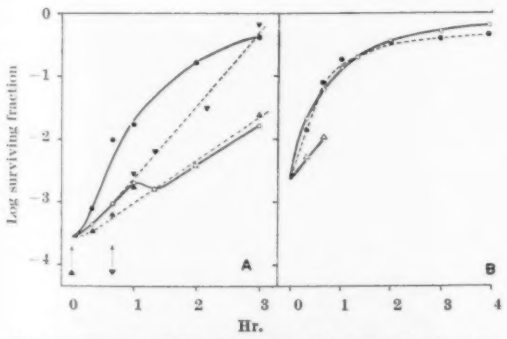

Fig. 1. Recovery of ultra-violet-irradiated *Escherichi coli B* in different media. Bacteria in late logarithmic phase of growth in *tris*–glucose–salt–casamino acid medium were irradiated for 30 sec. at 1 m. distance from Toshiba germicidal lamp (15 W.). *A*. Irradiated bacteria were shaken in *tris*–glucose–salt–casamino acid medium at 37° C. (—○—), with 20 μgm./ml. of chloramphenicol added to it immediately following irradiation (– –▲– –) or after 40-min. incubation (– –▼– –) as shown by the arrows, or in 0·1 *M* phosphate buffer (*p*H 7·0) + 0·02 *M* glucose (—●—). *B*. They were shaken in 0·1 *M* phosphate buffer (*p*H 7·0) + 0·02 *M* glucose with (– – ● – –) and without (—○—) 100 μgm./ml. of chloramphenicol, or in *tris*–glucose–salt–casamino acid medium (—△—)

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An Association between Capsulation and Phage Sensitivity in *Erwinia amylovora*

During a recent outbreak of fire blight in Britain[1,2] more than 150 isolations of the pathogen, *Erwinia amylovora*, have been made in this laboratory. On initial plating of infected material two types of colony are often observed[3]. The dominant type has characteristic markings on 'Yeastrel' peptone agar plates incubated at 30° C. For convenience, strains which form this type of colony are called 'typical'; those forming the second type of colony, which is relatively featureless, and less opaque, are called 'atypical'. Some strains are intermediate in character, even after re-streaking several times. With some such strains the opacity of individual colonies on a single streak plate may show considerable variation, with others, each individual colony appears mottled; marking may or may not be apparent on the more opaque colonies. Some 'typical' strains regularly yield a small proportion of unmarked colonies.

Using the wet indian ink method with a phase-contrast microscope, it was observed that the majority of cells of the 'typical' strains had small but distinct capsules when grown on either 'Yeastrel' peptone agar or a phage-test medium containing 1 per cent glycerol[3]. In contrast, some of the 'atypical' strains showed no capsules (Table 1). The remaining strains showed varying proportions of capsulated and non-capsulated cells. An accurate

Table 1. Colony Form, Capsulation and Phage Sensitivity of 28 Strains of *Erwinia amylovora*

Form of colony at 30° C. on 'Yeastrel' agar	No. of strains	Proportion of capsulated cells	Sensitivity to phages 4*L* and 4*S*	Sensitivity to phages 23
Typical	2	majority	*Ch*	—
Typical	4	majority	*Ch*	(*c*)
Intermediate	2	many	*Ch*	*C*
Intermediate	1	many	*ch*	*c*
Intermediate	3	few to many	*ch*	*C*
Atypical	4	few to many	*ch*	*C*
Atypical	1	rare	(*c*)*h*	*C*
Atypical	1	few	—	*C*
Atypical	8	rare	—	*C*
Atypical	2	none	—	*C*

Key: *C*, confluent lysis, little background turbidity; *c*, moderate background turbidity; (*c*) heavy background turbidity; *h*, halo

assessment was not possible with this method, because non-capsulated cells tended to clump in the indian ink though they were not agglutinated in normal saline. Also, some intermediate 'atypical' strains showed varying proportions on different occasions. An approximate estimate of the degree of capsulation of each strain was made and the results of two observations were recorded as follows in Table 1: majority (> 75 per cent), many (25–75 per cent); few (< 25 per cent); rare (< 1 per cent).

Crosse[1] has reported the isolation of phages which lyse *E. amylovora*. Since then, further phages have been isolated in this laboratory using the technique described by Crosse and Hingorani[3,4]. Two of these, 4*S* and 4*L*, give large (about 3·0 mm.) and small plaques (about 1·0 mm.) respectively on 'typical' strains. A third gives small plaques on 'atypical' strains.

The relationship between colony characteristics at 30° C., degree of capsulation and phage sensitivity with 28 strains is shown in Table 1. At one extreme there are the so-called 'typical' strains, the majority of the cells of which appear capsulated. These are lysed by phages 4*L* and 4*S*, which produce confluent lysis with a little background turbidity; the lysed area is surrounded by a distinct halo which extends on continued incubation. 'Typical' strains are not normally lysed by phage 23 or, if lysis is observed, it is largely obscured by background turbidity. At the other end of the scale are some of the stable 'atypical' strains which appear virtually non-capsulated or which at the most have rare capsulated cells. They are not lysed by phages 4*L* and 4*S*, but are strongly lysed by phage 23. Various intermediate reactions which have been observed with the remaining strains are also shown. Like capsulation, the reactions may vary slightly from one experiment to another, but the general picture remains the same.

It would appear from these observations that sensitivity to phages 4*L* and 4*S* depends on the presence of capsules and to phage 23 on the absence of capsules, and that where the culture contains a mixture of capsulated and non-capsulated cells some reaction with both phages can be expected. The strength of the reaction would seem to depend on the relative proportions of each; whether or not lysogenization occurs has not yet been fully investigated. Similar observations have been made by Rakieten, Eggerth and Rakieten[5] with mucoid strains of *Klebsiella*, *Escherichia* and *Aerobacter*. They found that phages acting on the mucoid strains were mainly type- or strain-specific. In my experience with *E. amylovora*, all strains giving 'typical' colonies (more than 130), whether isolated from *Pyrus*, *Crataegus*, *Sorbus* or *Cotoneaster* spp., also two strains in the National Collection of Plant Pathogens (100 and 311), have been sensitive to phages 4*S* and 4*L*.

The presence of extending halos in the case of reactions with phages 4*L* and 4*S* and capsulated strains suggests that an enzyme capable of hydrolysing capsules is produced[6,7].

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VIROLOGY

Filamentous Structures in Ectromelia Virus-infected Cells

The presence of parallel arrays of thin lamellæ clustered in bundles in the cytoplasm of some tumour cells containing Shope fibroma virus has been depicted by Bernhard *et al.*[1]. Fawcett[2] has observed irregular, coarse bundles of dense filaments in the cytoplasm of virus-containing tumour cells of the Lucké renal adenocarcinoma. Morgan *et al.*[3], reviewing their earlier micrographs of chick chorioallantois infected with fowl pox, found clusters of dense filaments, indistinguishable from those described by Bernhard, in the cytoplasm of ectodermal cells containing virus particles[4]. More recently, Reczko[5] has demonstrated the presence of groups of lamellæ in the epithelial cells of guinea pig peritoneum infected with swine pox virus. In electron micrographs of sections of chick chorioallantoic membranes infected with ectromelia virus, bundles of filaments have been observed in association with intra-cytoplasmic virus inclusion bodies.

Chorioallantoic membranes of 10-day-old White Leghorn embryos were inoculated[6] with a suspension of ectromelia virus so diluted as to give semiconfluent lesions after 72-hr. incubation at 36° C. The inoculum was second-egg passage material derived from infected mouse spleen originally received from Dr. W. C. Cutting of Stanford University. After incubation for 72 hr., membranes were fixed for about 5 min. while still in the eggs with buffered 1 per cent osmium tetroxide containing sucrose[7]. Membranes were excised and fixation continued for another 60 min. Ultra-thin sections of methacrylate-embedded tissue were examined with an RCA *EMU 2-B* electron microscope.

An infected mesodermal cell depicted in Fig. 1 shows a bundle of filaments with discrete virus particles interposed between it and a large virus inclusion body. A portion of the nucleus is seen in the lower left corner. Fig. 2 is a higher magnification showing in greater detail the fine structure of the filaments. The thickness of filaments is 8 mμ on the average, while the length is about 3–4μ. Virus forms, 265 × 200 mμ, with dense inner bodies lie between the bundle of filaments and inclusion body.

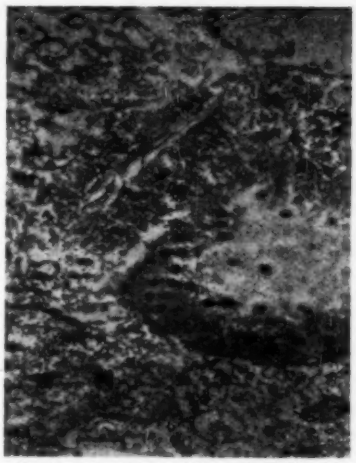

Fig. 1. Mesodermal cell of 72-hr. ectromelia virus-infected chorioallantois. A mitochondrion in a degenerative stage is seen immediately to the right of the nucleus (*N*). The filament bundle appears at the upper left. (In both Figs. 1 and 2, the scale mark represents 1μ.) ($\times$ *c.* 7,300)

the periphery of which is shown in the lower right corner.

In the numerous sections studied the filaments have generally been observed to be, at least topographically, in close association with virus forms and with inclusion bodies. In the case of Shope fibroma, the filaments, 7 mμ in diameter, were noted to be closely associated with cytoplasmic masses of virus or precursors of virus and were interpreted as possible by-products of a lipid nature of virus metabolism[1]. Fawcett observed typical virus particles contiguous to the filaments and believed that these might be related in some obscure way[2]. Reczko, noting the consistent location of these formations in the vicinity of developmental forms of the virus in the early stages of infection, concluded that these were probably connected with the process of virus synthesis[5].

The role of these filaments in virus development has not been established. Their presence in the cytoplasm of virus-infected cells has thus far been described for only the five aforementioned agents. It is possible that on careful study these structures will be observed in cells infected with other viruses[8]. Whether they be by-products of virus metabolism, pathological cellular responses to virus invasion, or stages in virus reproduction, will have to await further and more extensive investigation.

Fig. 2. Higher magnification of portion of infected mesodermal cell showing fine structure of filament bundle and viral forms (arrows) with dense inner bodies. ($\times$ 19,600)

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An Indicator Plaque-forming System for Demonstration of Interference by Non-cytocidal Strains of Rabies Virus

FIXED strains of rabies virus are known to multiply in chick embryo tissues[1,2]. So far, however, a cytocidal effect of the virus on monolayers of primary chick embryo cell cultures has not been observed. Since chick embryo-adapted strains of rabies virus interfere with infection by homologous virus in animal cells[3], the present investigations were undertaken to demonstrate interference of rabies virus with a superimposed infection of western equine encephalitis virus, which is known to produce cytopathic changes in these cultures.

Monolayers of chick embryo cells in 60-ml. Petri dishes were exposed for 2 hr. to $10^{6\cdot2}$ mouse *LD*50's of a fixed (*CVS*) strain of rabies virus in 0·2 ml. inoculum. 5 ml. of Eagle–Earle's medium were then added and the plates incubated at 37° C. for 24 hr. in an atmosphere of about 5 per cent carbon dioxide. The medium was discarded and the cells were exposed for 45 min. at 37° C. to western equine encephalitis virus. The plates, overlaid with neutral agar, were then incubated for 72 hr., and the cell sheets stained with a 1 : 10,000 solution of neutral red for the plaque count. Complete inhibition was observed of plaque formation by western equine encephalitis virus up to 2 million plaque-forming units, the highest concentration used. Essentially the same results were reproducible on repeated tests. In a preliminary study street virus also appeared to interfere with western equine encephalitis in the same system.

Quantitative studies of the rabies virus with the western equine encephalitis indicator system described gave promising results. Increasing dilutions of rabies virus showed a consistent titration pattern in repeated tests. Table 1 illustrates typical results obtained in one of the tests. In this table it will be noted that the 1 : 5,000 dilution of rabies virus, equivalent to about $10^{2\cdot5}$ *LD*50 in 0·2 ml. inoculum, still interfered with western equine encephalitis.

Although preliminary experiments indicate that the rabies virus particle itself may interfere with subsequent infection of the cell with western equine

Table 1. Interference Produced by Fixed Rabies Virus to Western Equine Encephalitis

Inoculum	No. of plaques per plate exposed to tenfold dilution of western equine encephalitis		
	10^{-4}	10^{-5}	10^{-6}
0·2 ml. of following dilutions of rabies virus* :			
1 : 1,250	0,0 (duplicate plates)	0,0	0,0
1 : 5,000	D†, D	27,10	4,9
1 : 20,000	D, D	104,137	31,23
Western equine encephalitis control	D	215,154	24,30

* Rabies virus inoculum undiluted contained $10^{7\cdot2}$ mouse *LD*50 per 0·2 ml.
† Degeneration of cells.

encephalitis, the interference can also be mediated in part by 'interferon' (as named by Isaacs and Lindermann[4]). This was demonstrated as follows. The culture medium covering the monolayer sheet exposed for 24 hr. to rabies virus was removed, centrifuged twice at 40,000*g* for 1½ hr., and the supernatant tested for absence of live virus by intracerebral inoculation into mice. This supernate, to which rabies antiserum was added to ensure neutralization of any residual live virus, was placed on fresh monolayers for 24 hr. The medium was then discarded and the cell sheets exposed to western equine encephalitis virus in the usual manner. Partial to complete inhibition of plaque formation by between 10 to 1,000 plaque-forming units of western equine encephalitis was obtained. This inhibition, however, was distinctly less than that observed with cell sheets directly exposed to rabies virus (Table 1).

The specificity of the test described was shown by neutralization of rabies virus with anti-rabies serum (Table 2). Increasing dilutions of anti-rabies serum against a constant amount of rabies virus displayed a titration pattern similar to that which occurred when serial dilutions of rabies virus alone were used as mentioned previously (Table 1).

Table 2. Neutralization of Constant Amount of Fixed Rabies Virus with Increasing Dilutions of Anti-Rabies Serum

Inoculum	No. of plaques per plate exposed to dilution of western equine encephalitis		
	10^{-5}	10^{-6}	10^{-7}
0·1 ml. rabies virus* + 0·1 ml. serum to given final serum dilution :			
1 : 2	44,54	3,5	0
1 : 20	81,92	9,18	1
1 : 200	88,84	18,6	0
1 : 2,000	0,0	0,0	0
Western equine encephalitis control	79,40	11,8	1,0

* Rabies virus inoculum contained $10^{7\cdot5}$ mouse *LD*50 per 0·1 ml. inoculum.

The sensitivity of the test compares favourably to that of the hæmagglutination test employed with myxoviruses. The ratio of the number of infective doses (egg *ID*50) per hæmagglutinating unit averages $10^{0\cdot3}$ for myxoviruses of high infectivity[5], as compared with $10^{2\cdot5}$ mouse *LD*50 of rabies virus being equivalent to approximately one interfering unit.

The results indicate the value of the interference system described for both quantitative and qualitative assays of a non-cytocidal virus and its specific antibody.

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Distribution-Free Interpretation of Spray-Drop Data

Spray-drop assay results do not always meet the Poisson-distribution test proposed by Williamson and Taylor[1], even though they may be valid and the sole product of expensive measurements. Williamson and Taylor have described an appropriate method for interpreting the spray-drop results obtained by the technique of Williams and Backus[2,3] for estimating the concentration of particles in a suspension. In this technique the unknown suspension is mixed with a suspension of polystyrene latex spheres in known concentration. The mixture is sprayed as a fine mist, and droplets are collected on specimen supports for examination by electron microscopy. For each droplet, the number of particles and the number of latex spheres observed are recorded. Counted for many droplets, these numbers constitute the raw data from which the concentration of particles in the unknown suspension is deduced.

A statistical analysis supplied by Williams and Backus was refined by Williamson and Taylor. This refinement included a test for the likelihood that the raw data represent samples from two Poisson-distributed populations, one for polystyrene latex spheres and one for the particles. If the raw data satisfy the Williamson and Taylor tests, and are free from induced variation arising from large variations in droplet size, then the ratio of the number of particles to the number of polystyrene latex spheres in the mixed suspension can be estimated from the ratio of the total particle-count in all droplets to the total count of polystyrene latex spheres in all droplets. The variance of this estimate can be calculated by the equation of Luria, Williams and Backus[4]. Lack of information on the precise distribution of the ratio precludes the possibility of using this variance to calculate a confidence interval. When data do contain variations induced by variation in droplet size, and do not satisfy the distribution test of Williamson and Taylor, distribution-free statistical methods may still be applied to obtain useful inferences about the concentration of particles in the unknown suspension. The use of order statistics is an appropriate distribution-free method in this instance. The following results, however, apply equally well to data which satisfy the requirements for the Williamson and Taylor analysis. In general, one would not expect the inferences of distribution-free analysis to be as precise as those obtained using the Williamson and Taylor analysis. The measure of central tendency is the median when distribution-free methods are used. The true population mean

Table 1

Drop No.	No. of particles (P)	No. of polystyrene latex spheres (PSL)	Ratio $\left(X = \frac{P}{PSL}\right)$	Order statistic
1	76	35	2·171	10
2	139	66	2·106	8
3	115	54	2·129	9
4	59	40	1·475	2
5	23	28	0·821	1
6	85	41	2·073	5
7	140	79	1·772	3
8	95	15	6·333	12
9	21	10	2·100	7
10	148	71	2·085	6
11	78	35	2·229	11
12	105	57	1·842	4

and median are equal only when the distribution is symmetrical.

Table 1 shows typical raw spray-drop data and the assignment of order statistics. The order statistics are simply integers assigned in increasing sequence to the calculated ratio X. Let n be the number of droplets the contents of which were counted. Let v be the true median of the population of possible ratios. Then the probability that the value of X with order statistic r is less than v, and the value of X with order statistic $n - r + 1$ is greater than v, can be calculated using equation 1:

$$(X_r < v < X_{n-r+1}) = \sum_{i=r}^{n-r} \frac{n!}{(n-i)!\,i!}\left(\frac{1}{2}\right)^n \qquad (1)$$

From this probability statement, confidence intervals for the median may be deduced. These confidence intervals are broader than those which might be obtained if the distribution of the ratio were known precisely. If needed, narrower confidence intervals for the same fiducial probabilities may be obtained by applying Fieller's theorem, which is discussed by Finney[6]. For much work in virus assay, confidence intervals based on equation 1 are sufficiently precise. Fig. 1 represents equation 1 in graphical form and shows, for each sample size, the value of r, which describes the confidence interval on the median.

To use Fig. 1, choose the line labelled with the required level of confidence. For the appropriate sample size, find r and compute $n - r + 1$. Locate the values of r and $n - r + 1$ in the column of order statistics assigned to the data. The data corresponding to these values represent the extremes of the confidence interval. For example, the median ratio for the data in Table 1 would have a 99 per cent confidence interval lying between 1·475 and 2·229 and a 95 per cent confidence interval between 1·772 and 2·171.

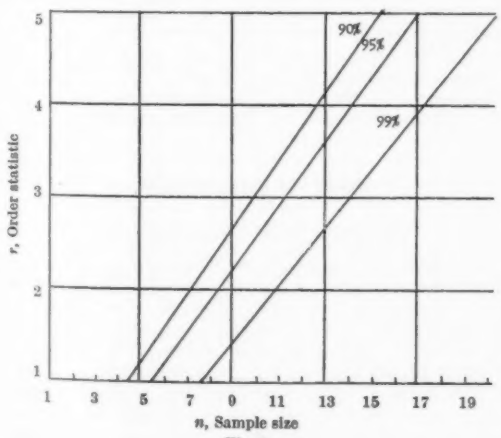

Fig. 1

When the underlying distributions for the data are certain, this information should be used in the analysis in order to discover any defects in the experimental technique, such as a bimodal distribution of drop-sizes. When order statistics alone are used in the analysis of spray-drop data, there is not the internal check for consistency provided by the Williamson and Taylor Poisson-distribution test. The distribution-free statistics are so convenient to use, however, that they can be recommended for broad application in situations where extreme precision in the estimate of the particle concentration is not required. The cost of this convenience may be the requirement for a better exercise of judgment in identifying valid data.

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SOIL SCIENCE

Swelling of Calcium Montmorillonite

WARKENTIN *et al.*[1], following up earlier work of Schofield[2], Bolt and Miller[3], and others, showed that swelling pressures developed by suspensions of sodium montmorillonite on an ultra filter could be predicted satisfactorily from a diffuse ion layer model based on classical Gouy–Chapman theory. Warkentin *et al.* also reported results of preliminary experiments with calcium montmorillonite for which the same model appeared to be inappropriate. A significant result was the decrease in magnitude of reswelling observed as the peak pressure applied to the sample increased. They concluded that forces not taken into account in the calculation must be present. Recently, Aylmore and Quirk[4], Greacen[5] and others have published discussions of these results which may now require reconsideration in view of further studies of calcium montmorillonite swelling.

Blackmore[6], working with calcium-saturated clay, was unable to duplicate the results of Warkentin *et al.*, and as a consequence obtained and re-examined one of the samples used in that study. In Fig. 1 are given some results of compression–decompression measurements on the Warkentin *et al.* sample together with a replot of results from Warkentin *et al.* and corresponding results from a freshly prepared sample. In these curves, the half-spacing is calculated as the quotient of the specific water content by the specific surface, a convention which assumes equal spacing between all clay particles according to the model which was found to be successful for clays saturated with monovalent ions. Apart from a half-spacing displacement for curves obtained with the old and new samples, current measurements on these samples

show very similar results and are consistent with more extensive results obtained by Blackmore, but the reswelling observed was less than half that reported by Warkentin *et al.*, except for samples which had experienced high pressures.

It was observed that the Warkentin *et al.* sample contained approximately 25 m.equiv. of sodium per 100 grams, due presumably to preparation by a calcium–resin column with insufficient free electrolyte[7]. This may account for the half-spacing displacement mentioned, but it apparently had little effect upon the magnitude of reswelling. No final solution of this anomaly has been achieved, but increasing evidence suggests that the swelling reported by Warkentin *et al.* is not that associated with a homoionic calcium montmorillonite.

Samples of calcium montmorillonite which had been equilibrated at various points in compression and decompression cycles were examined by X-ray diffraction and the presence of 18·8 Å. [001] spacings was demonstrated at all water contents and pressure equilibria. The average thickness of the 'packets' of particles presumed to be responsible for the diffraction peaks was estimated from the extent of line broadening. It was then possible to correct the swelling results for the presence of this 'condensed' phase and to estimate swelling between packets. It was also demonstrated that the exterior surface of the packets rather than residual single sheets not associated with packets was the active site of swelling.

Fig. 2 shows the original and corrected reswelling curves for a sample of calcium montmorillonite with 10^{-4} *M* electrolyte, and it is seen that agreement between the corrected curve and the curve obtained from the Gouy–Chapman model is surprisingly good. On the basis of this and related work it may be concluded that any swelling which occurs in a calcium clay–water system is largely of osmotic origin for the water contents studied, is a consequence of disorder within the sample, and can be approximated by calculations based on the Gouy–Chapman model.

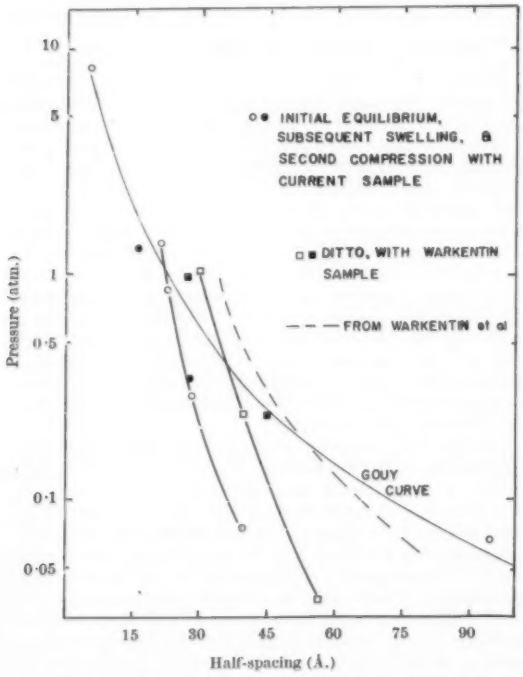

Fig. 1. Swelling pressure as a function of half-spacing for calcium montmorillonite with 10^{-4} *M* free electrolyte. Results are plotted for the theoretical curve, for a current sample, and for a repeat run with the sample used by Warkentin *et al.* in an earlier study, along with one of the re-swelling curves reported in this latter work

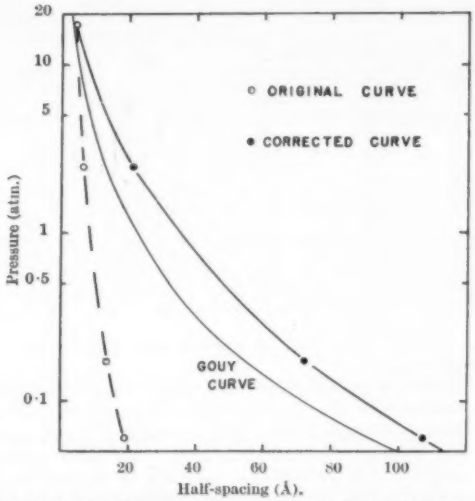

Fig. 2. Swelling pressures as a function of half-spacing for calcium montmorillonite calculated in the original form where half-spacing is obtained from total water and total surface area, and in the form corrected for 'packets' of unit sheets of clay. The average number of sheets per packet in this sample was 7, and the corrected curve has been displaced to the right by 3 Å. so that it will coincide with the uncorrected one at the point of maximum pressure

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ANTHROPOLOGY

Apidium and *Oreopithecus*

In view of attention recently directed to the taxonomic position of *Oreopithecus* among primates, as a result of new discoveries by Hürzeler[1] and Butler and Mills[2], a re-analysis of possible affinities of *Oreopithecus* with *Apidium*, from early Oligocene deposits of the Fayum, Egypt, seems desirable. Two fundamental points regarding this small Egyptian genus have been largely overlooked in recent literature on primate phylogeny. These are: first, that

Fig. 1. Above, *Oreopithecus bambolii*, type; size approx. × 2 (after Hürzeler, ref. 1); below, *Apidium phiomense*, type; size approx. ×4. Apices of principal cusps indicated in black

ranking *Apidium* as a primate is still justifiable, and second, that (among primates) its greatest resemblances are to *Oreopithecus* (Fig. 1). As both these assessments depend on partly qualitative taxonomic judgments, either may continue to be debated. In the following account the bases for these two conclusions are briefly outlined.

Osborn's reluctance to designate an ordinal position for *Apidium* in his description[3] of the type and only specimen was understandable in 1908. Much less was then known of *Oreopithecus* dental patterns, and little or nothing had been published regarding a number of early Tertiary primates, with which *Apidium* can now be compared. Such forms, since discovered, include *Parapithecus* and *Propliopithecus*, both from the same Oligocene fluviomarine formation which yielded *Apidium*. Direct observation of the type-specimens of these three genera shows that they are possibly inter-related. Although the teeth of *Apidium* are more cuspidate than are those of the other two Fayum mandibles, substantial agreements obtain among all three in conformation of major molar cusps and in other details. The suggestion by Osborn that *Apidium* might be allied to *Cebochoerus*, a primitive suid of the late Eocene of Europe, was rejected by both Schlosser[4] and Gregory[5]. Even so, Osborn's caution evidently has seeded an unnecessarily persistent doubt that *Apidium* can be given an accurate assignment to an order. Actually, a sound assessment should be possible. The teeth preserved are complex and little worn, so that, together with details of the jaw ramus, the occurrence and morphology of a great many separate features can be noted. Gregory[5] held that *Apidium* represents a plausible structural progenitor for the cercopithecoids, an assessment which still has some merits. He also stressed its close dental similarity to *Oreopithecus*. Since the latter was then regarded as a cercopithecoid monkey correct assessment was further complicated. Recently, Piveteau[6] and Hürzeler[1] have suggested that *Apidium* is a condylarth related to *Phenacodus*, but this is improbable (see Simons, ref. 7).

Among non-primates the closest similarities with *Apidium* are still to be seen in *Cebochoerus*, rather than with any Condylarthra. Nevertheless, these correspondences also appear to be superficial. There are a large number of proposed species of *Cebochoerus*, some of which have very little resemblance to *Apidium*. A specimen of *Cebochoerus* sp. at Princeton University, No. 11472, from the late Eocene phosphorites of south-central France shows more features of agreement with *Apidium* than most members of the genus do. Yet their differences are significant. Although a small individual of *Cebochoerus*, this jaw is almost twice as large as that of the Fayum primate, and, unlike it, has a progressive increase in depth of the horizontal ramus posteriorly, a feature common in artiodactyls, but rare in primates. Moreover, the principal molar cusps of the suid are higher and occupy a more central position on the teeth than they have in *Apidium*. In the latter the antero-internal margin of the mandible starts to turn inward toward the symphysis next to $P_{\bar{4}}$, thus suggesting a reduced number of teeth in the front of the jaw (as in catarrhines) and unlike *Cebochoerus*, other artiodactyls, and Condylarthra generally. While third molar heels in both are large, that of *Apidium* lacks the distinct separation of the hypoconulid lobe of $M_{\bar{3}}$ seen in *Cebochoerus* and typical of Artiodactyla. A raised area or centroconid is present in molars of both forms under consideration, but as this structure also occurs in the same position in *Oreopithecus* it need not indicate a phyletic connexion between *Apidium* and *Cebochoerus*. This suid also differs from *Apidium* and *Oreopithecus* in having a more pronounced constriction between trigonid and talonid of $M_{\bar{1}-\bar{3}}$ and a distinct crest joining hypoconid and hypoconulid.

In spite of this evidence, it cannot be denied that convergence between some primates and some suids can be very deceptive. As late as 1951, the view that *Oreopithecus* might be a suid was advanced in print. A similar confusion of these two groups lay behind the description of "Hesperopithecus". Now that Hürzeler, through recent collecting in Italy, has performed the valuable service of making *Oreopithecus* one of the better-known fossil primates (in terms of skeletal parts represented) its ordinal position is no longer in doubt. This problem resolved, the primate status of *Apidium* is practically confirmed by way of the following observed similarities with *Oreopithecus*.

In *Oreopithecus* the fourth premolar, $P_{\bar{4}}$, is two-cusped, while at best in *Apidium* it is only incipiently so. It must be assumed, however, that at some point in the ancestry of *Oreopithecus* $P_{\bar{4}}$ had only one principal cusp, as is typical of Eocene and earlier primates. Moreover, the multiplication of cusps in the talonid region of $P_{\bar{4}}$ in *Apidium* is reminiscent of the condition here in *Oreopithecus* and in some Eocene primates as well. A small internal cusp also may be the homologue in the Fayum form of the inner cusp of $P_{\bar{4}}$ in *Oreopithecus*. Occasionally in the latter the internal cusp of the trigonid is somewhat smaller and lower than the outer, so that the bicuspedness is not developed to the same degree in all specimens of the Tuscan primate.

Agreement in structure in the first molar tooth, as between the two species, is striking. In both forms the basal cingulum is most distinct around the anterior end of the tooth. The two also agree in the position and shape of the paraconid element, and the protoconid and metaconid of both exhibit three major crests running down from their apices, two of these being directed more or less antero-internally and the third running postero-lingually. They are alike in having the central cusp or centroconid less distinct here than in $M_{\bar{2}}$, but differ from *Cebochoerus* in which this eminence is ridge-like instead of being low and rounded. Possible homologies of this cusp are of some interest. In *Apidium* and *Oreopithecus* the centroconid occupies a position at about the centre of a cross drawn through protoconid, metaconid, hypo-

conid and entoconid. Among primates such a shape and location of the centroconid is limited to these two forms. In some fossil mammals, including the condylarth *Phenacodus*, there is a subsidiary cusp on the antero-internal slope of the hypoconid which has been called a mesoconid. A slight prominence here is sometimes present in *Pliopithecus* and *Gigantopithecus*, but in them the cuspule never has a completely central position. It seems improbable that the structures here termed mesoconid and centroconid are homologous. If they are, then the centroconid must have arisen by 'slipping' toward a central position along the antero-internal crest of the hypoconid after an initial subdivision of the latter had occurred. That this could happen is doubtful. A preferable assumption may be that the centroconid arose in the *Apidium–Oreopithecus* branch of the hominoids as a neomorph, and is not to be equated with the mesoconid seen in phenacodonts, hyopsodonts and several other archaic mammalian families. In *Apidium* the M_1 talonid is somewhat broader compared to the trigonid than in *Oreopithecus*, but this is true only for the M_1; both differ from *Cebochoerus*, which has broader trigonids than talonids. A rather broad and flat hypoconulid in *Apidium* is suggestive of an earlier condition of development of the large area seen here in *Oreopithecus*; there is already some thickening of the posterior basal cingulum of the hypoconid which, by joining the hypoconulid area, could produce the broad posterior molar shelf of *Oreopithecus*.

In addition to similarities noted for the first molar which are largely repeated here, second molars of both show considerable reduction of the paraconid element, so that it consists only of an anteriorly bowed ridge or crescent between the apices of proto- and metaconids. Each exhibits a more distinct centroconid and elsewhere a slight mesostylid cuspule. As might be expected for an earlier form, in *Apidium* M_2 is slightly less specialized than in *Oreopithecus*, and also shows similarities with M_2 of *Pliopithecus*, and *Gigantopithecus*.

The third molar is the largest and longest of the molar series in both primates (although in *Apidium* its full size is obscured by the fact that it has not fully erupted). In Fig. 1 the orientation of this tooth has been altered, so that its crown can be seen in the same plane as those of the remainder of the series. This illustration shows that in both species there is a large hypoconulid flanked laterally by accessory cuspules. A long postero-internal buttress of the hypoconid runs between ento- and hypo-conulid in the specimen of *Oreopithecus* figured here (after Hürzeler). This might be an important difference from *Apidium*, were it not for the fact that this ridge is missing in some specimens of the former.

The lack of connecting links, interposed temporally and morphologically between these two primates, *Apidium* and *Oreopithecus*, renders claims of an ancestor–descendant relationship between them uncertain. However, the resemblance between the two is striking considering that they are separated in time by more than twenty-five million years. *Apidium* appears to be a primate having particular significance in determining the time of origin of the Oreopithecidae. Fragmentary as they are, when taken together, the Fayum primates indicate the possibility that cercopithecoids, pongids and oreopithecids were already distinct in the early Oligocene. Accurate conclusions about the bearing of both *Apidium* and *Oreopithecus* on the question of hominid origins will have to await the discovery of additional specimens and completion of studies now in progress.

Elwyn L. Simons

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University of Pennsylvania, Philadelphia, 4.

[1] Hürzeler, J., *Abhandl. Schweizerischen Paläont. Gesell.*, **66**, 3 (1949); *Verhandl. Naturfor. Gesell. Basel*, **69**, No. 1, 1 (1958).
[2] Butler, P. M., and Mills, J. R. E., *Bull. Brit. Mus.* (*Nat. Hist.*), **4**, No. 1, 1 (1959).
[3] Osborn, H. F., *Bull. Amer. Mus. Nat. Hist.*, **56**, 253 (1908).
[4] Schlosser, M., *Beitr. Paläont. und Geol. Österreich-Ungarns*, **24**, 51 (1911).
[5] Gregory, W. K., "The Origin and Evolution of the Human Dentition" (Williams and Wilkins Co., Baltimore, 1922).
[6] Piveteau, J., "Primates, Paléontologie Humain", in "Traité de paléontologie", **7**, 1 (Paris, 1957).
[7] Simons, E. L., *Amer. Mus. Novitates*, No. 1976, 1 (1959).

MISCELLANY

"The Lunar Surface"

In proposing new, or partly new, ideas in a field where many conflicting opinions are held, one is always open to misinterpretation and, unfortunately, the review of my book "Strange World of the Moon", appearing in *Nature* of March 12, p. 718, although fair in intention, misrepresents some of the points I make.

Thus, Dr. Pagel states that 'lunavoes' "are essentially broken bubbles of gas bursting out from a contracting surface". This is true only with a considerable degree of poetic licence. Neither Spurr nor I have proposed such an idea. We interpret lunar craters as calderas of collapse, produced by the caving-in of laccolithic domes due to the withdrawal of magmatic support. Escape of gases plays an important part in the process, but the uplift is not a 'bubble'. Collapse calderas are well known to geologists and a classical study of these structures was published by Howel Williams in 1941.

Dr. Pagel states that "Mr. Firsoff attributes ray systems to erosion by running water". This is a very unlikely explanation and he appears to have confused my interpretation of the rays as ejected matter accumulated in hollows with Spurr's suggestion that the radial gullies on the lower slopes of such formations as *Autolycus* and *Aristillus* may be drainage channels produced by torrential downpours during copious eruptions of steam.

Omitting minor inaccuracies, I pass to Dr. Pagel's unexceptionable conclusion that the presence of water "on the lunar surface is highly implausible", which is precisely what I said. Liquid water could exist on the surface of the Moon only exceptionally, temporarily and in small quantities. My contention is that, surface rocks being specifically lighter than water, it may exist beneath the surface. Such investigation as I have made also appears to show that there is no physical reason for denying the possibility of local surface deposits of ice and snow surviving for long periods in certain circumstances. Photodissociation is discussed.

V. A. Firsoff

Mr. Firsoff's clarification of 'lunavoes' is very helpful, and I must apologize for having confused his explanation of rays with Spurr's explanation of radial gullies. My argument against the presence of water applies equally to ice and snow, which would be dissociated by solar ultra-violet radiation in the vapour phase.

B. E. J. Pagel

FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Tuesday, June 7

UNIVERSITY OF LONDON (at the Institute of Archæology, Inner Circle, Regent's Park, London, N.W.1), at 5.45 p.m.—Mr. G. J. Hurst: "Deserted Medieval Villages".* (Eighth of nine lectures on "Recent Archæological Discovery in Britain". Further lecture on June 14.)

Tuesday, June 7—Thursday, June 9

INSTITUTE OF PERSONNEL MANAGEMENT (at the Connaught Rooms, Great Queen Street, London, W.C.2), at 9.30 a.m. daily—Conference on "The Implications of Recent Research for Personnel Management".

Wednesday, June 8

UNIVERSITY OF LONDON (at King's College, Strand, London, W.C.2), at 5.30 p.m.—Dr. A. J. Dalton (National Cancer Institute, Bethesda, U.S.A.): "Electron Microscopic Studies on a Series of Plasma Cell Tumours in Mice".*

WOMEN'S ENGINEERING SOCIETY (at "Hope House", 45 Great Peter Street, London, S.W.1), at 7 p.m.—Annual General Meeting.

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Prof. J. B. Cragg: "Some South Atlantic Islands".

Thursday, June 9

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Mr. G. E. Cook, Mr. D. G. King-Hele and Mr. D. M. C. Walker: "The Contraction of Satellite Orbits Under the Influence of Air Drag"; Mr. F. L. Curzon, Mr. A. Folkierski, Mr. R. Latham and Mr. J. A. Nation: "Experiments on the Growth Rate of Surface Instabilities in a Linear Pinched Discharge".

UNIVERSITY OF LONDON (in the Physiology Theatre, University College, Gower Street, London, W.C.1), at 5 p.m.—Dr. S. J. Bach: "The Metabolic Fate of Protein-Nitrogen in the Mammal".*

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

ASSISTANT PROFESSOR, and a LECTURER IN THE PHYSICS DEPARTMENT—The Secretary, Heriot-Watt College, Edinburgh (June 9).

LECTURER (with research experience in experimental nuclear physics) IN PHYSICS—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (June 9).

RESEARCH ASSISTANT IN THE DEPARTMENT OF BOTANY, to assist in cytological studies in algae—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (June 9).

ASSISTANT, Grade B (graduate, preferably possessing a special degree in geology, with ancillary botany and ecology), IN THE NATURAL SCIENCE DEPARTMENT, to teach geology and biological subjects—The Principal, Derby and District College of Technology, Kedleston Road, Derby (June 11).

ASSISTANT LECTURER, Grade "B", IN BIOLOGY, for work at G.C.E. ("O") and G.C.E. ("A") standard—The Clerk to the Governors, North Staffordshire Technical College, Stoke-on-Trent, Staffs (June 11).

ASSISTANT LECTURER IN THE DEPARTMENT OF PHYSIOLOGY—The Registrar, The University, Liverpool, quoting Ref. CV/N (June 11).

ASSISTANT LECTURER or a LECTURER IN THE DEPARTMENT OF BOTANY (with special reference to taxonomy); and a RESEARCH DEMONSTRATOR IN THE DEPARTMENT OF BOTANY—The Registrar, University College, Singleton Park, Swansea (June 11).

LECTURER IN PHYSIOLOGY—The Registrar, The University, Manchester 13 (June 11).

LECTURERS IN PHYSICS; a LECTURER IN THEORETICAL PHYSICS; and an ASSISTANT LECTURER IN PHYSICS—The Registrar, The University, Manchester 13 (June 11).

RESEARCH ASSISTANT (graduate with a higher degree and special interests in instrumental astronomy) IN ASTRONOMY in the Department of Physics of the Faculty of Science—The Registrar, The University, Manchester (June 11).

RESEARCH ASSISTANT (with a degree in genetics or in botany, biochemistry or microbiology with an interest in genetics) to Prof. D. Lewis, F.R.S., in the Department of Botany—The Secretary, University College, Gower Street, London, W.C.1 (June 11).

PRINCIPAL LECTURER (with a degree and research experience in pharmacy, extensive experience in the teaching of all branches of pharmaceutics, and preferably industrial pharmaceutical experience) IN PHARMACY—The Registrar, Bradford Institute of Technology, Bradford 7 (June 15).

LECTURER IN CHEMICAL ENGINEERING—Prof. D. M. Newitt, F.R.S., Department of Chemical Engineering and Chemical Technology, Imperial College of Science and Technology, South Kensington, London, S.W.7 (June 18).

LECTURER (with a special interest in cartography) IN GEOGRAPHY—The Secretary of University Court, The University, Glasgow (June 18).

LECTURERS (2) IN PHYSICS—The Registrar, The University, Hull (June 18).

TEMPORARY LECTURER IN PURE MATHEMATICS—The Registrar, University College, Singleton Park, Swansea (June 18).

LECTURER IN HYDRAULICS AND HYDROLOGY IN THE DEPARTMENT OF CIVIL ENGINEERING—The Registrar, King's College (University of Durham), Newcastle upon Tyne 1 (June 20).

ASSISTANT IN RESEARCH (with an appropriate honours degree) IN ANIMAL PHYSIOLOGY—The Secretary, School of Agriculture, The University, Downing Street, Cambridge (June 22).

LECTURER or ASSISTANT LECTURER IN BIOLOGY, BOTANY or ZOOLOGY, to take the subject to pass degree standard; a LECTURER IN CHEMISTRY; and a LECTURER IN MATHEMATICS—The Principal, Bede College, Durham (June 25).

RESEARCH STUDENTS IN PHYSICS, for work in the following topics: magnetic resonance of the solid state, gas discharge studies, investigations of defects in glass and other fibres, and studies on the properties of oxide cathodes—The Registrar, University College of North Staffordshire, Keele, Staffs (June 25).

SENIOR RESEARCH ASSISTANT (with an honours degree, preferably in metallurgy or alternatively in chemistry, and some postgraduate or industrial research experience) IN THE DEPARTMENT OF METALLURGY, to investigate a problem in the field of electrodeposition—The Registrar, The University, Nottingham (June 25).

CHAIR OF EDUCATION in the University of New England, Armidale, New South Wales, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, June 27).

THERMAL SYNDICATE RESEARCH FELLOW IN THE CERAMICS DIVISION of the Houldsworth School of Applied Science, for research on the physics of silica glass—The Registrar, The University, Leeds 2 (June 28).

LECTURER IN PLANT PATHOLOGY (VIROLOGY) at Waite Agricultural Research Institute, University of Adelaide, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, June 30).

RESEARCH FELLOW IN CHEMISTRY, for work on the structures of physiologically active polypeptides—The Secretary, The University, Exeter (June 30).

ASSISTANT LECTURER or LECTURER (preferably with research experience in genetics) IN THE DEPARTMENT OF BIOLOGY AS APPLIED TO MEDICINE—The Secretary, Department of Biology as Applied to Medicine, Middlesex Hospital Medical School, London, W.1 (July 10).

IMMUNOLOGIST (medical graduate interested in tumour immunology) to develop a Section of Immunology in the Division of Pathology; an ELECTRON-MICROSCOPIST (graduate in medicine with experience in pathology or an honours science graduate with histological experience) to be trained as electron-microscopist in the Division of Pathology; a BIOCHEMIST (honours graduate) for the Cyto-Chemistry Section of the Division of Pathology; and a BIOCHEMIST (honours graduate with research experience in lipid biochemistry) in the Division of Chemistry and Biochemistry—The Director of Research, Imperial Cancer Research Fund, Lincoln's Inn Fields, London, W.C.2 (July 15).

LECTURER (with a higher degree, preferably a doctorate of philosophy, with some research and teaching experience) IN CHEMISTRY at the University of Queensland, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, July 15).

SENIOR LECTURER or LECTURER IN BOTANY (PLANT PHYSIOLOGY) at Victoria University of Wellington, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand and London, July 15).

LECTURER (with an honours degree in philosophy, and a special interest or qualification in either logic or the philosophy of science or the history of ideas) IN PHILOSOPHY at the University of Canterbury, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand and London, July 18).

SENIOR LECTURER IN PHYSICAL CHEMISTRY, and a LECTURER (with special interests in inorganic chemistry) IN CHEMISTRY at the University of Hong Kong—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Hong Kong and London, August 31).

FIELD ASSISTANT (female, with an honours degree in geography, preferably with subsidiary geology and/or botany or zoology) at Malham Tarn Field Centre, near Settle, Yorks—The Secretary, Field Studies Council, 9 Devereux Court, Strand, London, W.C.2.

GRADE B ASSISTANT (graduate, preferably with industrial and/or research experience) IN PHYSICS—The Principal, Medway College of Technology, Horsted, Maidstone Road, Chatham, Kent.

LECTURERS (graduate metallurgists or physical chemists interested in extraction metallurgy, chemical metallurgy or metallurgical analysis) IN METALLURGY—The Secretary, Sir John Cass College, Jewry Street, London, E.C.1.

PHYSICAL CHEMIST, Scientific Officer grade, to study the persistence and toxicity of residual films of insecticides—The Secretary, Rothamsted Experimental Station, Harpenden, Herts.

PLASTICS CHEMIST or PLASTICS TECHNOLOGIST (preferably with appropriate research or industrial experience) IN THE DEPARTMENT OF MATERIALS, to assist in the organization and supervision of the teaching laboratories and to participate in the research activities of the plastics group—The Recorder, The College of Aeronautics, Cranfield, Bletchley, Bucks.

POSTDOCTORATE FELLOW (with a Ph.D. degree (or equivalent) of a recognized university) IN BIOCHEMISTRY in the Psychiatric Research Unit situated in the University Hospital, Saskatoon, to carry out fundamental research into some of the biochemical aspects of mental illness—The Personnel Officer, Saskatchewan Department of Public Health, Health and Welfare Building, Regina, Saskatchewan, Canada.

RESEARCH ASSISTANT (with a first degree of the University of London and an interest in ultrasonics and electronics) IN ULTRASONIC PHYSICS—The Principal, Kingston Technical College, Fassett Road, Kingston-upon-Thames, Surrey.

RESEARCH FELLOW (Senior or Junior) (with the degree of Ph.D., and preferably experience in research involving proteins) IN THE DEPARTMENT OF BIOLOGICAL CHEMISTRY, for work with Dr. J. L. Simkin on biosynthesis of proteins—Dr. J. L. Simkin, Marischal College, The University, Aberdeen.

SCIENCE GRADUATE (male or female) IN THE LABORATORIES OF THE ABERDEEN AND NORTH-EAST SCOTLAND BLOOD TRANSFUSION SERVICE

—The Regional Director, Aberdeen and North-East Scotland Blood Transfusion Service, Royal Infirmary, Aberdeen.

SCIENTIFIC OFFICER (honours graduate in chemistry or biochemistry) IN THE RADIOBIOCHEMISTRY DEPARTMENT, for biochemical research involving the use of radioactive isotopes, and in particular C^{14} and tritium—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Reading, Berks, quoting Ref. 60/3.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

International Tin Research Council. Annual Report 1959. Pp. 36. (Greenford, Middx.: Tin Research Institute, 1960.) [163

Polymer: The Chemistry, Physics and Technology of High Polymers, Vol. 1, No. 1 (March 1960). Pp. 124. Published quarterly. Annual Subscription including postage—U.K. 100*s*.; U.S.A. 15 dollars. (London: Butterworths Scientific Publications, 1960.) [163

University of Cambridge. Report of the Observatories Syndicate for the year ending 1959 September 30. Pp. 5. (Cambridge: The University, 1960.) [163

Planning, Vol. 26, No. 440 (14 February 1960): Challenge and Change—Papers from the CED Conference. Pp. 65–104. (London: Political and Economic Planning, 1960.) 4*s*. [163

Royal Society of Arts. Industrial Art Bursaries Competition—Report for 1959. Pp. 56. (London: Royal Society of Arts, 1960.) [163

The Registrar General's Statistical Review of England and Wales for the Three Years 1954–1956. Supplement on Mental Health. Pp. viii+198. (London: H.M. Stationery Office, 1960.) 11*s*. 6*d*. net. [163

Government of Northern Ireland: Ministry of Agriculture. Leaflet No. 15: Suitable Varieties of Fruit. Pp. 15. Leaflet No. 43: Tuberculosis in Poultry. Pp. 3. Leaflet No. 86: Manuring Farm Crops. Pp. 15. Leaflet No. 140: Fowl Typhoid. Pp. 3. (Belfast: Ministry of Agriculture, 1960.) [163

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The Year Book of the Royal Society of London, 1960. (No. 64.) Pp. 302. (London: The Royal Society, 1960.) 21*s*. [163

Air Ministry: Meteorological Office, Professional Notes No. 124: The Preparation of Statistical Wind Forecasts and an Assessment of Their Accuracy in Comparison with Forecasts made by Synoptic Techniques. By C. S. Durst and D. H. Johnson. Pp. 27. (M.O. 623*d*.) (London: H.M. Stationery Office, 1959.) 1*s*. 9*d*. net. [183

Commonwealth Mycological Institute. Mycological Papers, No. 75: A Revised List of Plant Diseases in Tanganyika Territory. By Dr. E. A. Riley. Pp. ii+42. (Kew: Commonwealth Mycological Institute, 1960.) 10*s*. [183

Government of Northern Ireland: Ministry of Agriculture. Leaflet No. 11: Fruit Tree Pests—Aphids and Apple Sucker. Pp. 7+2 plates. (Belfast: Ministry of Agriculture, 1960.) [183

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